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9885

Effects of Thyroid, Dinitroörthocresol on Urine Output of Thyroidectomized Dogs with Moderate Diabetes Insipidus.

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In an earlier report[1] we raised the question whether the ability of thyroid to render effective the diuretic action of anterior lobe is specific or is due only to its effect on general metabolism. This matter has been investigated as follows: Two dogs were rendered polyuric by hypothalamic puncture; they were thyroidectomized at

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[1] White, H. L., and Heinbecker, Peter, *Am. J. Physiol.*, 1937, **118**, 276.

20 and at 30 days after puncture. In both cases daily urine output fell to about 40% of its prethyroidectomy level but was still about 3 times normal. On two occasions with each dog desiccated thyroid (0.1 g per kilo per day) was given for a 7- to 10-day period; on all 4 such occasions daily urine output increased 2 to 3 fold.

Not enough determinations of the effect of this dose of thyroid on the basal metabolic rate were made to permit a definite quantitative statement. There were considerable variations in results but our observations justify the statement that such dosage for such a period elevates oxygen consumption by not more than 50% and probably considerably less. The dosage may be continued for even longer periods without any toxic symptoms, significant loss of weight or increase in urine output in the normal dog. The above statement is borne out by the findings of the much more extensive series of determinations by Kunde.[2] She found that 0.25 g desiccated thyroid per kilo daily produced an increase in BMR of 33% on the fifth day and 50% on the seventeenth day. In another of her experiments 0.4 g per kilo daily had the same effect, *i.e.*, 30% increase on the fifth and 50% on the seventeenth day.

Our BMR determinations were carried out with a modified Roth-Benedict spirometer, using a metal cone and heavy rubber dam mask, with dead space reduced to a minimum. Our average on 3 normal dogs is 850 calories/M^2/24 hours, with a probable error of about 10%. We have not obtained the high degree of reproducibility attained by Kunde[3] and by Kunde and Steinhaus;[4] such reproducibility requires long and careful training and was not considered essential to our purpose.

We next established the dosage of dinitroörthocresol required to produce approximately a 50% elevation of BMR in normal dogs. This was found to be 1 mg per kilo 3 times daily at 8-hour intervals. On such a regime, basal oxygen consumption determined at various times of day was increased on the average about 50%, with variations from +20 to +70. If the diuretic effect of the dosage of thyroid employed is due merely to its effect on metabolic rate, our dosage of DNC should have a similar diuretic effect. No diuretic effect was obtained, however, in 3 periods of 6 days on the 2 operated dogs. The results on 1 dog are shown in Fig. 1.

In these dogs thyroidectomy resulted in an immediate drop in

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[2] Kunde, M. M., *Am. J. Physiol.*, 1927, **82**, 195.

[3] Kunde, M. M., *J. Metab. Res.*, 1923, **3**, 399.

[4] Kunde, M. M., and Steinhaus, A. H., *Am. J. Physiol.*, 1926, **78**, 127.

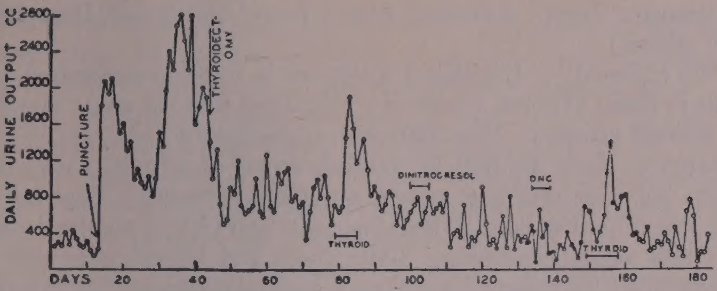

FIG. 1.

Effect of thyroidectomy and of thyroid and dinitrocresol administration on daily urine output of polyuric dog.

urine output, although not to the normal level; in the course of a few months the output slowly declined to normal. The transitory polyurias of hypophysectomy (removal of anterior lobe, pars intermedia, infundibular process and part of stalk) or of stalk section are usually immediately abolished by thyroidectomy. The high and permanent polyurias of complete hypophysectomy, including pars tuberalis and median eminence, may be unaffected by thyroidectomy (unpublished observations). We believe that the extent of the effect of thyroidectomy on the polyuria is proportional to the residual amount of pitressin-forming tissue.

Summary. The diuretic action of thyroid synergistic with anterior lobe is probably not due to its effect in elevating metabolic rate, as is indicated by the finding that a dosage of DNC producing a similar elevation of BMR has no diuretic effect.

9886

Adrenal Weights in Tumor-Bearing Rats.*

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The adrenal cortex has repeatedly been shown to be implicated in response of the body to toxins and infections. (Lewis,[1] Perla and

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* Aided by a grant from the International Cancer Research Foundation.

[1] Lewis, J. T., *Am. J. Physiol.*, 1923, **64**, 506.

Gottesman,[2] Biebl,[3] Zwemer,[4] Bracci-Torsi,[5] Baker and Hinmann,[6] and others.)

We reported[11] a diminished resistance in hypophysectomized animals to doses of cobra venom and postulated that the effect was due to adrenal atrophy. We attempted at the time to increase the resistance of these animals by administration of adrenal cortex extracts but were unable to obtain satisfactory results. This failure was due in part at least to the very acute and crucial manifestations of cobra venom poisoning. Later Perla and Rosen[7] were able to demonstrate the restorative action of adrenal cortex extract in hypophysectomized animals subjected to histamine poisoning.

In a cancer experiment involving intact and hypophysectomized animals bearing Walker No. 256 tumors, it was noted that the adrenals enlarged in the intact tumor-bearing animals above the normal size, while in the hypophysectomized animals the usual adrenal atrophy was present. The enlargement of adrenals in the intact animals is presumably either a result of absorption of toxic products of tumor necrosis or of the changed general metabolism of the body. Tumor necrosis was present also in the tumors of the hypophysectomized animals but the adrenals were not enlarged. The increased size can not then be due to a simple direct action of the toxins on the adrenals. The findings suggest that the pituitary is essential to this type of compensatory enlargement. Table I presents the values obtained.

TABLE I.
Adrenal Weights in Male Rats.

	No.	Body Wt	Wt of Adrenals, g
Normal	27	165 ± 4.5	.0301 ± .0007
Intact, with Walker No. 256 tumors	14	159 ± 3.5	.0560 ± .0023
Hypophysectomized	13	131 ± 3.2	.0148 ± .0007
Hypophysectomized, with Walker No. 256 tumors	10	161 ± 1.7*	.0160 ± .0006

*Weights before operative procedures.

Histological studies indicate that the enlargement of the adrenals in the intact tumor-bearing animals is not due to the myeloid infiltrations reported by McEuen and Selye[8] and Lewis[9] to accompany this tumor, but consists primarily of an increase in the cytoplasm and number of cortical cells and an increase in lipoid content. This would correspond to the "prolonged activity" type of Zwemer.[4]

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[2] Perla, D., and Gottesman, J. M., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 474.

[3] Biebl, M., *Z. f. d. ges. exper. Med.*, 1933, **91**, 662.

[4] Zwemer, R. L., *Am. J. Path.*, 1936, **12**, 107.

[5] Bracci-Torsi, C., *Gior. d. med. prat.*, 1936, **18**, 59.

[6] Baker, D. D., and Hinmann, E. H., *J. Trop. Med.*, 1936, **39**, 117.

[11] Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 26.

[7] Perla, D., and Rosen, S. H., *Arch. Path.*, 1935, **20**, 222.

Higgins and Cragg[10] have reported hyperplasia of the adrenals similar to that observed by us in rats chronically poisoned with carbon tetrachloride. This was associated with atrophy of the gonads. These authors were not able to determine from their experiments whether this was due to a pituitary effect or to direct toxic actions. If the effect is similar in origin to ours we must assume that pituitary secretion is involved.

Conclusion. The presence of the hypophysis is necessary to adrenal hypertrophy in animals bearing Walker No. 256 tumor. This finding suggests that the pituitary may be essential to any adrenal cortex hypertrophy. Either certain toxins or metabolic processes sensitize the adrenal tissues to the pituitary adrenotropic hormone or they act directly on the hypophysis itself to cause an increased output of this substance. Work is now in progress to determine which process is involved.

9887 P

Atrophy of the Thymus in Normal and Hypophysectomized Rats Following Administration of Cortin.

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Moon[1] noted atrophy of the thymus in rats following administration of the adrenotropic principle of the anterior lobe of the pituitary glands. His observations have been confirmed by Lyons and Simpson of the same laboratory.[2]

This investigation deals with the effect on the thymus of administering massive amounts of cortin to normal rats and to rats which had been hypophysectomized. Forty male rats, each having an initial body weight of 180 g, were used. Ten normal animals were untreated and 10 normal animals each received 10 cc of cortin daily in their drinking water (each cubic centimeter of cortin represented 75 g of adrenal glands). Twenty hypophysectomized rats each received an amount of adrenotropic hormone daily which previous assay had proved adequate to maintain the adrenal cortex in hypophysectomized rats. This adrenotropic hormone was prepared and furnished through the courtesy of Dr. H. D. Moon. Ten of the hypophysectomized animals were given 10 cc of cortin daily in their drinking water; the remaining 10 did not receive cortin. In this experiment the intake of food of the 2 groups of hypophysectomized rats was regulated in order to equate the loss of weight. It was necessary to restrict the intake of food in the case of the rats which did not receive cortin to a definitely smaller amount than was consumed by those which did receive cortin. At the end of 7 days all of the animals were killed for necropsy by exsanguination.

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[8] McEuen, C. S., and Selye, H., *Am. J. Med. Sci.*, 1935, **189**, 423.

[9] Lewis, M. R., *Am. J. Cancer*, 1937, **30**, 95.

[10] Higgins, G. M., and Cragg, R. M., *Proc. Staff Meet. Mayo Clinic*, 1937, **12**, 582.

[1] Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 34.

[2] Evans, H. M., personal communication to the author.

The administration of large amounts of cortin causes marked involution of the thymus in the intact rat. The loss of weight which occurred in these animals cannot be satisfactorily accounted for by the small reduction in the intake of food. Similarly, in the hypophysectomized rat in which the adrenal cortices have been maintained at normal size with adrenotropic hormone, additional treatment with cortin hastens atrophy of the thymus (Table I).

TABLE I.
Atrophy of Thymus Following Administration of Cortin.

		Body wt, g		Thymus wt, mg	
	No. of rats	Avg.	Range	Avg.	Range
Normal, no cortin	10	196	184-210	377.1	325-485
Normal, 10 cc of cortin daily	10	151.7	142-162	53.7	40-76
Hypophysectomized, 0.5 cc adrenotropic hormone daily, no cortin	10	141.4	136-147	187.5	140-280
Hypophysectomized, 0.5 cc adrenotropic hormone daily, 10 cc of cortin daily	10	137.7	125-143	49.4	39-64

9888 P

Factors Affecting Oxidation-Reduction Potentials Produced by Bacteria in Synthetic Media.

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Measurements of the apparent oxidation-reduction potentials established in a synthetic medium by the growth of *Escherichia coli* showed that many irregularities were encountered which were not present when determinations were made in peptone nutrient broth cultures of the same organism. It is the object of this paper to present a study of the factors involved in these irregularities. The elimination of such would make it possible to obtain consistent data from which valuable conclusions might be deduced.

A vacuum tube null-point instrument drawing a maximum current of 10^{-11} ampere was utilized in making the physical measurements. No polarization phenomena were observed and the apparatus gave calculated values for easily polarizable half cells. Bright platinum electrodes were used. A liquid medium made by weighing the requisite C.P. or analytical reagents and adding to redistilled water allowed the test organism, *Escherichia coli,* to grow vigorously. This basic solution, a slight modification of that of Koser and Saunders,[1] was composed of Na_2HPO_4 1.4 g, KH_2PO_4 1.0 g, NaCl 2.0 g, $MgSO_4$ (anhy.) 0.1 g, l-asparagine 3.0 g, l-tryptophane 0.2 g, d-glucose 2.0 g, and redistilled water 1 liter. Sterilization was accomplished by filtration. The final pH was 6.9. A nutrient broth medium used for comparison was composed of beef extract (Swift) 3.0 g, peptone (Difco) 10.0 g, NaCl 5.0 g, and redistilled water 500 ml. This was adjusted to a pH of 6.9, sterilized in the autoclave, and to it was added 500 ml of sterile phosphate buffer (pH 6.9 at 37.5 C). The buffer was present in a final concentration of M/30.

Culture vessels consisted of 180 ml electrolytic beakers stoppered with rubber stoppers containing appropriate holes for the electrodes, for a stirring apparatus, for the agar-KCl bridge and to allow for gas exchange. The medium, previously inoculated with *E. coli,* was pipetted into the electrode containing vessels after the latter had been sterilized in the autoclave. Vessels were incubated in a water bath at 37.5C±0.02C.

The basic synthetic medium gave very irregular results when incubated under stationary conditions. Irregularities appeared between the values obtained for like duplicate vessels and between duplicate electrodes in the same vessel. The Eh intensity levels in the synthetic medium when read by means of platinum gauze, foil, or wire electrodes were dependent upon the type of electrode used. This is demonstrated in Fig. 1. These phenomena, apparently not due to acid production, did not appear in broth media.

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[1] Koser, S. A., and Saunders, F., *J. Infect. Dis.*, 1935, **56**, 305.

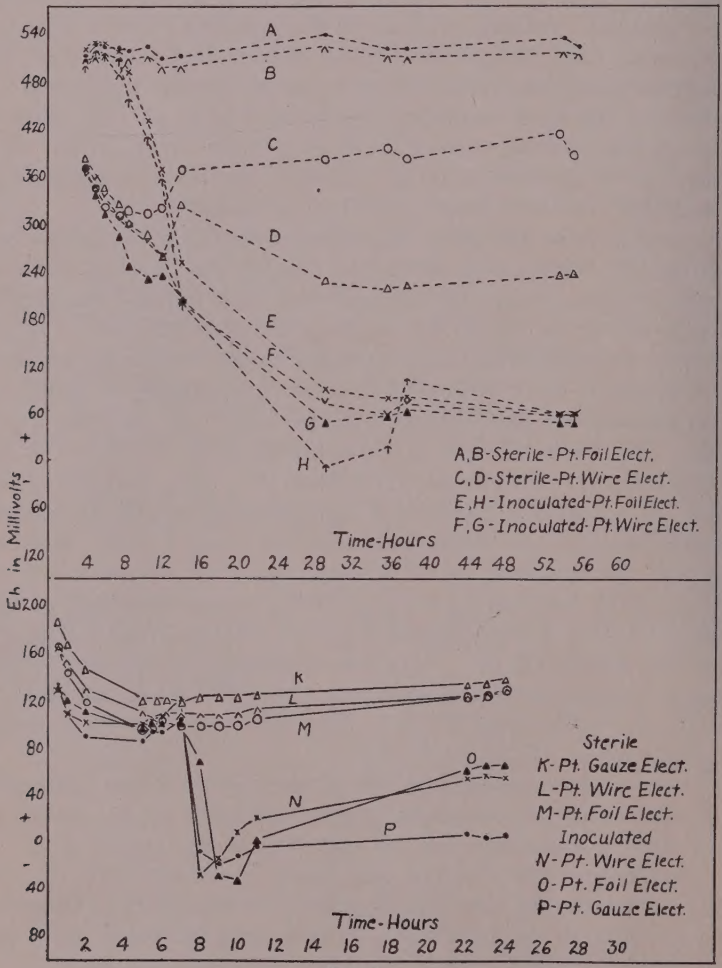

FIG. 1.

Time-potential curves obtained in the basic synthetic medium under aerobic (- - - -) and anaerobic (——) conditions. Each curve represents an average of the readings obtained from duplicate electrodes in the same vessel.

When purified nitrogen was bubbled through cultures of the basic medium irregularities were absent for the first seven hours of incubation (lower portion of Fig. 1).

Thus it would appear that the potentials that have ordinarily been attributed to the production of measurable oxidation-reduction systems by growing organisms are to a certain extent dependent upon the presence of poising materials present in the medium before growth is initiated. Such a poising agent can act as a mediator between the bacteria and their systems and the electrodes. Preliminary experiments have supported this concept. Stratification of growth has been shown to occur in some media and it seems that the more comparable results obtained by bubbling nitrogen or other gases through a medium might in part be due to agitation in addition to the removal of oxygen electrode effects. Simple mechanical agitation introduces complications because of the oxygen lability of the systems present.

9889 P

Antisera for Organ-Proteins.

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In none of the experimental work conducted in this laboratory could tissue-proteins be completely freed from blood proteins, nor has it been found possible to separate the blood proteins quantitatively from the autolyzed tissue-protein. So, antisera to tissue-protein or to autolysates always contained precipitins for blood proteins. Absorption *in vitro* with blood proteins usually removed not only their precipitins but also any precipitins that may have been formed for tissue-proteins. One investigator, Gilman,[1] working with urinary protein from a patient with severe chronic nephritis, was able to remove quantitatively the precipitins for hemoglobin, pseudoglobulin, and albumin by absorption *in vitro,* and obtained a precipitin that was specific for renal protein as found in an autolysate of the kidney When the renal autolysate was used for immunization, absorption *in vitro* of the blood-protein precipitins removed all of the precipitins.

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[1] Gilman, G., *J. Urology,* 1935, **34**, 727.

Gay and Rusk[2] " . . . obtained some evidence that the critical reinjection of horse serum causes a temporary fall in precipitin content." Hektoen and Welker,[3] working with pure antigens, were able to remove certain specific precipitins by intravenous injection of corresponding antigens without affecting other precipitins present in the immunized rabbit.

Fresh human organ-tissue was ground in a meat chopper, washed several times in saline in an attempt to wash out as much of the blood proteins as possible, and then ground for 48 hours in a ballmill with saline and toluene. The tissue was again washed in saline in the centrifuge until freed from practically all water-soluble protein. The finely divided tissue was strained, fixed on aluminium cream, and injected into the leg and thigh muscles of rabbits—about 3 cc per depot until 30 cc had been injected into each animal.

In 10 to 14 days precipitins for hemoglobin, pseudoglobulin, and albumin were found in high titer. Equal parts of 1% human hemoglobin, 1% human pseudoglobulin, and 1% human albumin to make 10 cc were then injected into the marginal ear vein of these animals. In 8 hours the precipitins for albumin had disappeared; in 10 hours the precipitins for hemoglobin and pseudoglobulin were also gone. Blood was drawn from the rabbit at 6-hour intervals and the serum tested for the above precipitins. In 36 hours after the injection the precipitin for hemoglobin had reappeared and in 42 hours those for pseudoglobulin and albumin were present. Serum collected earlier, when free from blood-protein precipitins, gave positive precipitative reactions with hepatic autolysates.

From rabbits injected with ground liver and kidney, the precipitins for blood proteins disappeared after 4 to 8 weeks, but the serum still reacted with autolyzed kidney and liver. This was not true in all the rabbits injected. In some, the interval was considerably longer and in one the precipitins for the organ-proteins disappeared with the precipitins for the blood proteins.

Fresh extracts, prepared by grinding the tissues in a mortar with sand and saline, were not specifically precipitable until the tissues had autolyzed. Autolyzing (37°C) liver tested at one-hour intervals for 4 hours and at 4-hour intervals for the next 48 hours, gave positive reactions, except the autolysate at the end of the first hour. Kidney-extract tested at 6, 12, 18, 24, 48-hour intervals gave positive reactions with kidney antiserum. Both systems cross-reacted. The liver antiserum precipitated with autolysates of adrenal, pancreatic, splenic, and thyroid tissue; brain-tissue was negative. Kidney-antiserum, tested with these autolysates, gave negative or unsatisfactory results. Only very low dilutions of the renal autolysates were reactive but precipitation occurred in high dilutions (1-100,000) of the hepatic autolysate.

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[2] Gay and Rusk, *Univ. California Publ. Path.*, 1912, **2**, 59.

[3] Hektoen, L., and Welker, W. H., *J. Inf. Dis.*, 1935, **57**, 337.

Summary. Precipitins for blood proteins disappeared from the circulating blood of rabbits immunized with renal and hepatic tissue as soon as these antigens are exhausted from the injected-tissue depots. The exhaustion of the tissue-proteins usually takes a much longer time. This provides a method for the preparation of tissue-antisera. Extracts of fresh liver and kidney do not give precipitative reactions with the antisera, but the autolyzed products of these organs do. Liver-autolysate seems to have an antigenic factor in common with the autolysate of kidney, pancreas, spleen, adrenals, and thyroids. Kidney-autolysate has an antigenic factor in common with liver but not with any of the other above-mentioned organ-autolysates.

9890

Effect of Inorganic Phosphorus on Bone Growth and Repair.

C. O. Adams. (Introduced by L. R. Dragstedt.)

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That the feeding of inorganic phosphorus will produce transverse lines or bands of increased density in the metaphyses of growing long bones has been demonstrated by means of roentgenograms by Phemister[1] and subsequently by various observers. This "phosphorus band" has been thought to be a "growth arrest line," but the hypothesis has not been proved. Such lines have been observed only where longitudinal growth has taken place. They are not produced by supplements of Vitamin D without phosphorus.

We observed in the roentgenograms of a few young patients who were receiving phosphorized cod liver oil, dense new bone about areas of healing fractures or osteotomies of long bones. The callus about the osteotomy site, along the edges of the chisel cut, appeared to show an increase in density similar to the phosphorus lines in the metaphyses of the same bones.

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[1] Phemister, D. B., *J. A. M. A.*, 1918, **70**, 1737.

These observations suggested the possibility that phosphorus might be causing an increased density in the new bone that was being formed through callus, and hence that such bands were not limited to the phenomenon of long bone growth. Such a possibility could be explained upon a basis of retardation of the formation of provisional callus, stimulation of osteogenesis, or excess deposition of inorganic phosphorus similar to retention of lead in the bones of patients suffering from lead poisoning. The demonstration of these transverse phosphorus lines at the site of fractures or osteotomies of growing bones also reopened the question of whether or not some longitudinal growth of long bones might occur from the cartilaginous callus of the healing fracture. Because of interest in these questions, which had been stimulated by the aforementioned observations, the following experiment was undertaken:

Twenty-nine rabbits from 5 litters, 8 to 12 weeks old, and 2 adult rabbits from a sixth litter were used. The right tibia of each animal was fractured in mid-shaft and immobilized in a plaster cast for 2 weeks. Half of the rabbits of each litter were given from .0006 to .003 g of inorganic phosphorus in the diet daily. Four litters received this supplement in the form of phosphorized cod liver oil mixed with their food. Two litters were given daily a pill of yellow phosphorus. When .003 g of phosphorus in cod liver oil was mixed with the daily food ration, the rabbits refused to eat. Roentgenograms of both lower extremities were taken each week. An animal and its control mate were killed each week after fracture and the entire tibia prepared for microscopic study.

By the end of the second week, transverse lines of increased density could be demonstrated by the roentgenograms, in the juxta-epiphyseal regions, of all of the animals receiving phosphorus, except the adult rabbit. These lines also were present in the microscopic sections. Similar transverse lines did not appear at the site of the healing fractures. There was no clinical, roentgenographic, nor microscopic evidence that the feeding of phosphorus had any effect on the rate of healing, the quantity or quality of callus formed. There was no evidence in the roentgenograms that any longitudinal growth of bone occurred from the cartilaginous callus of the healing fractures.

Measurements of the normal tibias showed an average decrease in rate of longitudinal growth of the bones of the animals receiving phosphorus, as compared with those on the stock diet without this supplement. This observation would seem to support the theory that the so-called phosphorus band is, in reality, a "growth arrest line."

The results of this experiment and reëvaluation of the earlier roentgenograms seem to justify the conclusion that the lines of increased density which we observed in the roentgenograms at the site of repair of fractures, osteotomies or bone defects simply represented formations of cortical bone and had no definite relation to the phosphorus the patients were receiving. They were not growth or growth arrest lines.

Conclusions. 1. In the experimental animals, the feeding of inorganic phosphorus in doses large enough to cause transverse metaphyseal lines, has no demonstrable effect on the roentgenographic or microscopic appearance of the healing of fractures. 2. Transverse bands of increased density which may be produced at the juxta-epiphyseal region of growing children or animals by the feeding of phosphorus are probably "growth arrest lines" similar in their etiology to those produced by acute diseases such as scarlet fever.

9891 P

Increase of Bactericidins in the Serum of Cattle Following Recovery from Infection with *Brucella abortus*.*

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Results given previously[1] have shown that bovine serum displayed a greater bactericidal activity against *Brucella abortus* than did a comparable amount of whole blood. Trials have accordingly been made to determine whether an increase in the titer of the serum bactericidins was to be found in individuals which had recovered from an infection with this organism.

The technic employed was essentially the same as that previously described.[1, 2] The serum was taken from (a) cows which had previously been artificially infected with *Br. abortus*, and from whose blood practically all agglutinins for the organisms had disappeared (*i.e.*, "recovered") and (b) from other animals which, insofar as was known, had had no contact with the organism (*i.e.,* "normal").

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* Paper No. 231 from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, in cooperation with the Bureau of Animal Industry, U.S.D.A. Published with the approval of the Director of the Station.

[1] Irwin, M. R., Beach, B. A., and Bell, F. N., *J. Inf. Dis.*, 1936, **58**, 15.

[2] Shrigley, E. W., and Irwin, M. R., *J. Immunol.*, 1937, **32**, 281.

A uniform quantity (0.3 cc) of serum, or of dilutions of serum, from an individual was added to each of a series of small tubes into which had been placed 0.05 cc of decimal dilutions of a 36- or 48-hour culture of a standardized saline suspension of *Br. abortus.* To each of the tubes containing diluted serum was then added 0.2 cc of complement of beef serum. (The complement was obtained by absorbing beef serum, with heat-killed cells of *Br. abortus,* at 0°C for 20-30 minutes.) Following incubation for 22-24 hours, the contents of each tube were poured into a petri dish with fluid pork-infusion agar, and incubated 5 or 6 days. For the bacterial control, 0.05 cc of each of the different dilutions was mixed with the fluid medium and poured into petri dishes. In these tests the number of organisms in the final dilution (*−7*) was generally between 10 and 50. Heated serum (0.3 cc), complement (0.3 cc), and the combination of heated serum (0.3 cc) and complement (0.2 cc), respectively, were treated in the same manner as the serum and diluted serum above. The results of this set-up, in contrast to the bactericidal activity of undiluted serum, provided a check on the efficiency of the absorption-technic.

Heated serum alone showed no bactericidal action, nor did complement by itself. Heated serum plus complement, however, was practically equal in activity to the undiluted, untreated serum, from "normal" or "recovered" animals, which allowed growth only in the *−2* or −3 dilution and beyond representing the destruction of from 10^4 to 10^5 organisms. Some activity was usually displayed by the serum of "normal" animals at a dilution of 1 in 25, rarely at the next dilution, 1:125. In contrast, however, serum from "recovered" animals regularly caused a reaction in dilutions of 1 in 625, occasionally at higher dilutions. These latter animals had been artificially infected[2] (by way of the eye) 18 to 20 months before these tests were made, hence the possibility of a higher titer at an earlier time, in relation to the infection, cannot be excluded.

These findings indicate the presence of "immune" bactericidins, following an infection, in the absence of an appreciable titer of agglutinins. Further tests on these, and on the fluctuations of the titer of bactericidins in the serum of normal individuals from time to time will be presented later, with a discussion of their possible significance and their relation to the immune opsonins reported by Huddleson.[3]

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[3] Huddleson, I. F., Johnson, H. W., and Meyer, D. B., Tech. Bull. No. 149, Mich. Agric. Exp. Sta., 1936.

9892

Lack of Refractoriness to Prolonged Thyrotropin Administration in Birds.

DOROTHEA STARBUCK MILLER. (Introduced by Emil Witschi.)*

From the State University of Iowa.

It has been reported by many investigators that prolonged injection of thyrotropic hormone induces refractoriness in the response of the mammalian thyroid. The initial stimulation of the glands is followed by a regression in all the indications of hyperthyroidism. Within 2 to 3 weeks the hyperplastic thyroids and the elevated metabolic rate return to normal.

In a preliminary series of thyrotropin injections in the English sparrow, the symptoms of hyperthyroidism apparently persisted over a period of 6 weeks. Therefore 2 additional groups of sparrows were injected continuously for longer periods to test whether the thyroids would become refractory. The thyrotropic fraction used was a benzoic acid precipitate of a pyridine extract of dry anterior pituitary of sheep, prepared according to the method of Greep.[1]

The first group of 3 female and 2 male sparrows were injected daily or every other day from January 10 to March 23, a 10-weeks period. The second group of 3 females and 3 males received injections from October 18 to March 10, a period of 20 weeks. As criteria of thyrotropic activity, the condition of the thyroids and elevation in the metabolic rate were considered.

Size of the Thyroids. The thyroids of all the birds killed after continuous thyrotropin administration were considerably enlarged. In Fig. 1 are shown camera lucida drawings of the thyroids of a control female, a female after 4 injections, and a female injected over a 10-weeks period.

Histological Findings. The enlarged thyroids show a high degree of hyperplasia characteristic of the stimulated gland. The photograph in Fig. 2 shows a thyroid section from a female sparrow killed after 10 weeks' injection, which may be compared with the control gland in Fig. 3.

Metabolic Rate. The basal metabolic rate, measured on a Bene-

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Professor Emil Witschi.

[1] Greep, R. O., *Am. J. Physiol.*, 1935, **110**, 692.

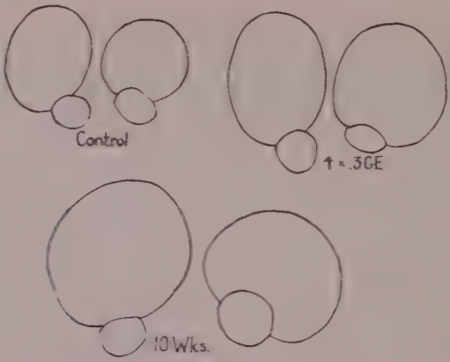
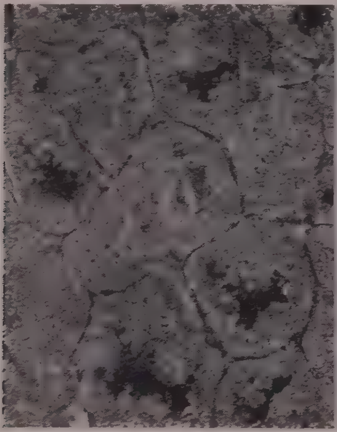


FIG. 1.

Camera lucida drawings of thyroids of female sparrows. Upper left, control; upper right, after 4 injections of thyrotropin: below, after thyrotropin injection for 10 weeks. × 10.



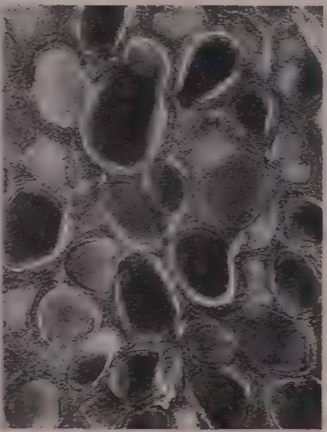


FIG. 2.

Section of sparrow thyroid, preserved after 10 weeks' injection with thyrotropin. × 360.

FIG. 3.

Section of thyroid from control sparrow. × 360.

dict closed-circuit apparatus,[†] remained elevated throughout the experimental period. The results of these determinations are presented in Table I. Each value is based upon 2 experimental birds and 2 or more controls. The percentage elevation in metabolic rate is the increase over that of the controls used in that particular run. Since the average deviation for all controls is ±3%, the elevated metabolic rate observed in the experimental birds is significant.

[†] The apparatus was kindly placed at our disposal by the Physiology Department of the University of Iowa, College of Medicine.

TABLE I.
Elevation in Metabolic Rate in English Sparrows Receiving Continuous Injections of Thyrotropin.

	Date	Injection period, wks	% elev. in MR
1st Group	3-3-37	7½	+20.30
(begun 1-10-37)	3-15-37	9	+17.09
2nd Group	11-2-37	2	+16.14
(begun 10-18-37)	11-23-37	5	+15.75
	3-10-38	20½	+12.86

These results indicate that the sparrow thyroid does not become refractory to continued pituitary administration. Greep, using the same thyrotropic preparation, reported that during continuous injections, the guinea pig thyroid returns to normal within 4 weeks. Evidently the bird is peculiar in failing to show this type of immunity reaction.

Summary. In the English sparrow, prolonged injection of sheep thyrotropic hormone does not lead to the development of refractoriness. The thyroids remain enlarged and hyperplastic, and the elevation in metabolic rate is maintained over periods up to 5 months.

9893

Testes and Hypophyses in Gassed Male Rats.

CHARLES A. PATTERSON, ERMA SMITH* AND A. D. PICKETT.
From the Department of Zoology, Iowa State College, Ames, Iowa.

A degree of chronic carbon monoxide asphyxia in man is frequent since daily inhalation of small amounts of illuminating gas is common experience. Williams and Smith[1] have shown typical asphyxial blood changes, decrease in body weight, and sterility to occur in male rats treated daily with illuminating gas. The present study concerns testicular and hypophyseal changes.

Eighty-eight male albino rats, Ames-Wistar strain, were arranged in 2 groups of 44 each, litter mates being equally distributed. A closed circuit respiratory apparatus into which illuminating gas could be measured directly from the city main was used; one group was exposed daily for an hour to 1.43% gas-air mixture. The mixture contained 0.34% CO.† Immediately after the hour of exposure the mean COHb was 50% and the blood sugar 132.0±1.6 mg %; 24 hours after exposure the mean COHb was 5% and the blood sugar 100.1±.05 mg %.

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* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Williams, I. R., and Smith, Erma, *Am. J. Physiol.*, 1935, **110**, 611.

After periods of treatment, ranging from a minimum of 50 to a maximum of 131 days, and 24 hr after the last gassing interval the rats were killed by decapitation. Eight hypophyses were prepared for cytologic study by the method of Severinghaus.[2] The testes were removed and weighed. Hanging drop preparations from the epididymides were examined for motility and number of spermatozoa.

TABLE I.
Testes.

No. of rats	Mean wt, g, right and left testes and epididymides	% of body wt	% decrease	Spermatozoa, motility and no.	Mating history
Control 44	4.28 ± .05	1.55		Myriads, actively motile	+
Gassed 44	2.01 ± .04	0.8	46	None or a very few sluggishly motile, or non-motile	—

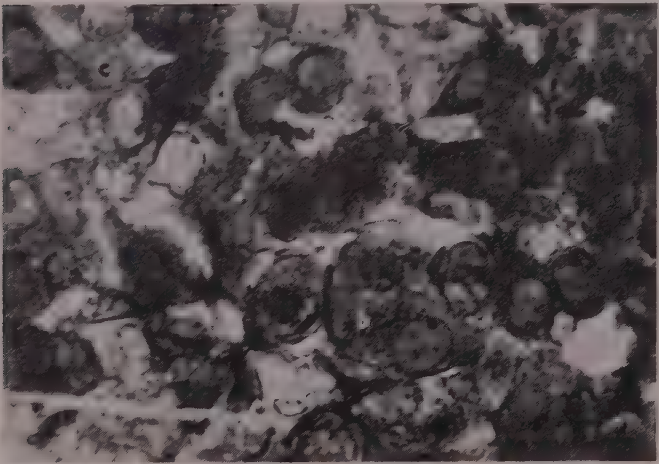

FIG. 1.
Photomicrograph of section of hypophysis from normal ♂ rat × 1180. b = basophile; c = chromophobe; e = eosinophile.

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† For other constituents of the gas see [1].

[2] Severinghaus, Anna E., *Anat. Rec.*, 1932, **53**, 1.

The mating history was negative in rats exposed to gas for 50 or more hours. Frequently no sperm could be seen in the hanging drop preparations from gassed rats, but prepared slides consistently gave evidence of spermatogenesis. If sperm were present usually they were nonmotile. The testicular size decrease is shown in Fig. 3.

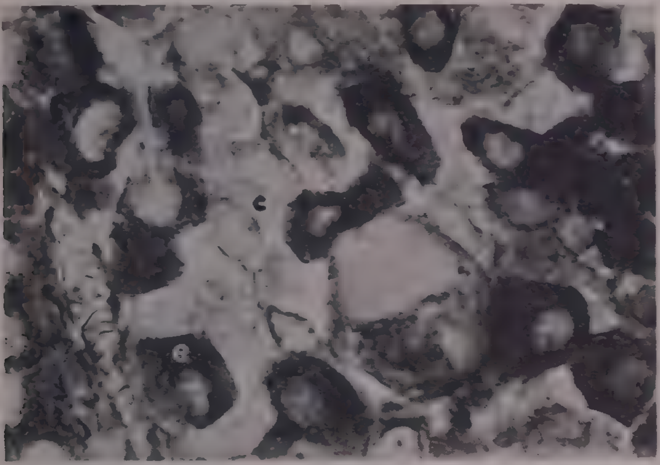

FIG. 2.

Photomicrograph of section of hypophysis from gassed ♂ rat × 1150. b' = **vacuolated basophile; c = chromophobe; e = eosinophile.**

Hypophyses. Figs. 1 and 2. Vacuolated and enlarged basophiles characterize the microscopic structure of the hypophyses in gassed rats. The mean diameters of 20 vacuolated basophiles were 23.83 μ by 15.35 μ: vacuole diameters 13.89 μ by 8.89 μ: nonvacuolated basophile diameters 20.9 μ by 10.9 μ. The mean diameters of nonvacuolated basophiles of the controls were 16.6 μ by 10.9 μ. Acidophile diameters did not differ in control and gassed animals.

The above closely resembles the hypophyseal changes described by Addison[3] as induced by castration. Since the basophiles are considered to be the cells which produce gonadotropic hormones, biologic assay of the hypophyseal hormones in the sterile gassed rats was the next procedure. The response of immature female rat ovaries and uteri was used to measure the gonadotropic potency.

Hypophyseal Hormone. Fig. 3 and Table II. When 36 of the male rats above described were decapitated, the entire hypophyses were removed aseptically, weighed in sterile containers, macerated by means of glass rods, and injected subcutaneously into immature

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[3] **Addison, W. H. F., *J. Comp. Neurol.*, 1917, 28, 441.**

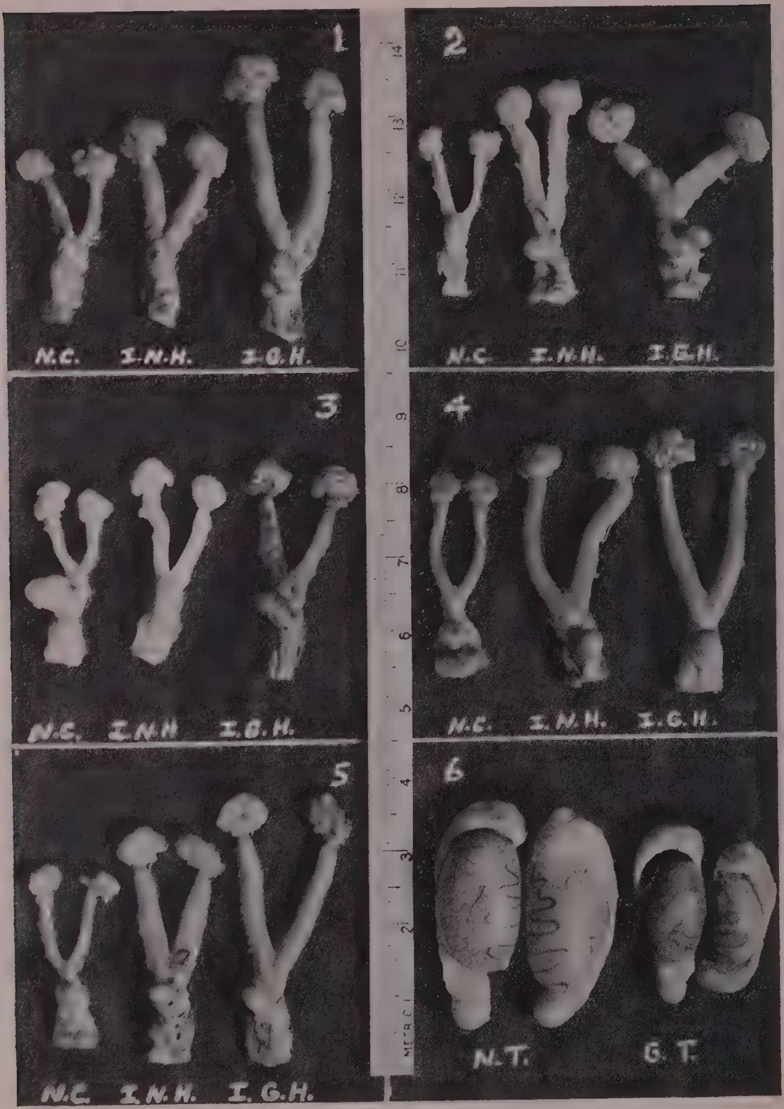

FIG. 3.

1, 2, 3, 4, 5, Ovaries and uteri of ♀ test rats. N.C., noninjected control; I.N.H., control injected with six hypophyses from normal ♂ rats. I.G.H., test ♀'s injected with six hypophyses from gassed ♂ rats. 6, Testes. N.T., normal rat; G.T., gassed litter mate. (Actual size.)

TABLE II.
Hypophyseal Hormone.*

	Series	Hypophyses total wt in mg 6 ♂'s†	♀ rats age, days	♀ rats wt, g	Ovaries wt, g‡	Uteri wt, g‡
Control ♀'s	1		26	65	19.2	36.1
Non-injected	2		23	38	14.7	23.7
N.C.	3		23	39	16.4	30.0
	4		25	72	31.1	57.3
	5		26	42	16.1	29.6
	Total		123	256	97.5	176.7
	Mean		24.6	51	19.5	35.3
Control ♀'s	1	45.4	26	66	68.1	101.0
Injected with	2	45.7	23	41	47.9	76.2
6 hypophyses of	3	38.9	23	41	34.0	64.1
ungassed ♂ rats	4	41.7	25	57	60.4	104.3
I.N.H.	5	41.9	26	43.5	64.3	127.0
	Total	213.6	123	248.5	274.7	472.6
	Mean	42.7	24.6	49.7	54.9	94.5
Test ♀'s	1	48.1	26	65	79.7	146.0
Injected with	2	46.9	23	45	95.0	130.5
6 hypophyses of	3	46.9	23	41	77.5	87.2
gassed ♂ rats	4	40.9	25	59	110.8	129.5
I.G.H.	5	38.2	26	44	80.3	129.2
	Total	221.0	123	254	443.3	622.4
	Mean	44.2	24.6	50.8	88.6	124.5

*Weights on preliminary group not included because the tissues were fixed in Bouin's fluid for slides.

†Hypophyses weighed without fixation.

‡Ovaries and uteri fixed in formalin before weighing.

female rats (Fig. 3, I.G.H.) 2 each day for 3 successive days. A control injected similarly with hypophyses from ungassed males (Fig. 3, I.N.H.) and a noninjected littermate control (Fig. 3, N.C.) were run simultaneously. The female rats were autopsied 96 hours after the first injection. The assay was conducted in six series.

Fig. 3 presents positive visible evidence of greater enlargement of ovaries and uteri in all rats injected with hypophyses of gassed rats. Calculations from Table II show the ovarian increase to be 1.61 times and the uterine increase 1.32 times that in the controls injected with hypophyses from ungassed rats.

The mean hypophyseal weight in ungassed rats was 7.12 mg; in gassed rats 7.36 mg. The mean difference is 0.25 mg; each female injected with hypophyses of gassed rats thus received 1.5 mg more gland. Heller, Lauson, and Severinghaus[4] have shown 1.5 mg of hypophysis to produce no significant ovarian increase but positive uterine enlargement. The consistent ovarian and uterine enlargement in our experiments proves a greater hormone content weight for weight in hypophyses of gassed rats.

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[4] Heller, Carl G., Lauson, Henry, Severinghaus, Elmer L., *Am. J. Physiol.*, 1938, **121**, 364.

Conclusions. 1. The combined testis and epididymis weight in rats is reduced about half after 50 or more one hour exposures to illuminating gas. The number of spermatozoa is greatly decreased and motility in the few viable sperm is impaired. 2. The hypophyses of gassed male rats become highly basophilic and many vacuolated castrate cells appear. 3. The gonadotropic hormone content of the hypophyses of gassed rats is increased.

9894 P

Myotonia Congenita in the Goat.

GEORGE F. SUTHERLAND AND QUIN F. CURTIS. (Introduced by H. S. Liddell.)

From Cornell University, Ithaca, N. Y.

In the course of conditioned motor reflex experiments on goats, we had the opportunity of observing so-called "stiff-legged" goats. Four such animals were furnished us through the kindness of Mr. D. E. Motlow, of Lynchburg, Tennessee.

It has been noted for years that animals of this strain, when startled, stiffen and fall over, remaining immobile for some seconds in what at first appears to be a general tonic spasm. Accordingly, they have commonly been labelled "nervous," "stiff-legged" or "epileptoid" goats.

Our investigations show, however, that this seizure is not a general tonic spasm produced by a special type of stimulus, but is caused by the failure of individual muscles to relax normally when strongly stimulated after a period of rest.

Stimulation of the motor points of the thigh with an adequate faradic current produces, not a simple twitch, but a tetanic contraction of the individual muscle or muscle group, with relaxation in from 5 to 30 seconds. On successive stimulation, the period of relaxation is progressively shortened until it approaches the normal twitch. Percussion of the muscle elicits a myotonic contraction of a few fibers, with characteristic slow relaxation.

These symptoms are accentuated by cold and by prostigmin (Roche), and are relieved by adequate doses of quinine.

These findings, together with the hereditary aspect of the anomaly, lead us to believe that we are dealing with Myotonia Congenita in the goat.

9895

Ineffectiveness of Sulfanilamide in the Treatment of Trichiniasis in Rats.

O. R. McCoy.

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Since the demonstration that sulfanilamide is an effective therapeutic agent in the treatment of infections caused by the coccal group of bacteria, many tests have been made of its possible value in the treatment of other diseases of varied etiology, particularly those for which no specific drug is known. So far, sulfanilamide has not been found of value in infections caused by bacilli, filterable viruses,[1] or animal parasites.[2] In view of the belief that the drug may act to increase host-resistance and to neutralize toxins, it was thought worthwhile to test its value in the treatment of trichiniasis, a disease in which the symptoms are mainly the result of toxemia, and for which there is no known effective drug treatment.

Two experiments were performed in which full-grown rats were infected by stomach tube with known numbers of *Trichinella spiralis* larvae, obtained free from infected rat muscle by artificial digestion.[3] In each experiment, one group of rats were given treatment with sulfanilamide (para-amino-benzene-sulfonamide), while another group were kept as controls. The dosage of sulfanilamide was the same as that employed by Gross and Cooper[4] for the treatment of pneumococcal infections in rats, namely, 125 mg per day. The drug was ground to a fine powder, suspended in a 10% solution of gum acacia, and given to the animals by stomach tube. Details of administration are presented in Table I. Usually, treatment was begun on the day after infection, at a time when the adult worms were developing in the intestine, and was continued during the period that the young larvae were developing in the muscles.

From 1 to 2 months after infection, all the animals were killed, their carcasses were digested individually, and a count was made of the total number of larvae which had developed in the muscles. The average amount of infection, as measured in this way, was approxi-

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[1] Dickerson, V. C., and Whitney, L. F., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 263.

[2] Keil, E., *Arch. f. Schiffs. u. Trop. Hyg.*, 1936, **40**, 400.

[3] For methods see McCoy, O. R., *Am. J. Hyg.*, 1931, **14**, 484.

[4] Gross, P., and Cooper, F. B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 225.

TABLE I.
Rats Infected with *Trichinella spiralis* and Treated with Sulfanilamide.

Group	No. of rats	No. of larvæ fed	Treatment with sulfanilamide	Avg No. of larvæ in muscles
Experiment 1				
Treated	4	2,000	125 mg for 12 days beginning the 6th day after infection	110,900
”	4	2,000	125 mg for 16 days beginning the day after infection	99,800
Control	8	2,000	None	93,200
Experiment 2				
Treated	9	3,000	125 mg for 12 days beginning the day after infection	165,600
Control	8	3,000	None	236,600

mately the same in the treated rats as in the control animals (Table I). In other treated rats (not listed in the table), killed at intervals after the beginning of treatment, no lethal effect was observed either on the adult worms in the intestine or on the developing larvae in the muscles. Also, as judged by the amount of weight lost during the course of the infection, the drug had no effect on the severity of the symptoms. In fact, the treated rats lost considerably more weight than the control animals, and also more than other rats which were given the same amount of drug but were not infected with *Trichinella*. No evidence was found to suggest, therefore, that sulfanilamide had any value in the treatment of experimental trichiniasis in rats.

9896

Human Ascitic Fluid as a Blood Substitute in Experimental Secondary Shock.

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From the Department of Pathology, University of Tennessee, and Department of Surgery, George Washington University.

The value of a substitute for whole blood for clinical and experimental use is self-evident. Various solutions have been utilized, such as blood plasma,[1, 2] gum-saline,[3, 4] gelatin-saline,[5, 6] hemoglobin-

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[1] Guthrie, C. C., and Pike, F. H., *Am. J. Physiol.*, 1907, **18**, 14.

[2] Richet, C., and Brodin, P., *Compt. Rend. Acad. Sci.*, 1917, **167**, 55.

[3] Morawitz, P., *Beitr. Chem. Physiol. Path.*, 1906, **7**, 153.

[4] Robertson, O. H., and Bock, A. B., *Rep. Med. Res. Council, Lond.*, 1918, No. 25, 213.

[5] Hogan, J. J., *J. A. M. A.*, 1915, **64**, 721.

[6] Clark, G. W., *J. Immunol.*, 1918, **3**, 147.

Ringer's solution,[7] and whole blood obtained from the placenta,[8] umbilical cord,[9] and cadaver.[10] In this study we have endeavored to determine the effectiveness of human peritoneal and pleural transudates as substitutes for blood in experimental hemorrhagic shock. In most of these experiments ascitic fluid (peritoneal transudate) was used. This was obtained from human patients suffering from cardiac decompensation, or from portal cirrhosis of the liver. The fluids were removed with aseptic precautions and filtered through fine gauze. Each fluid was submitted to the following examinations: Kahn test, blood agar culture and chemical determination of the protein and electrolyte content. The fluids were stored at a temperature of 0C to 5C for varying periods up to 5 months. No preservatives were used and bacteriological examinations were made at intervals using the blood-agar plate method. It was found that such fluids could be kept sterile for long periods, and that prolonged refrigeration did not affect their efficiency as substitutes for blood.

Chemical Composition. In a series of ascitic fluids, quantitative chemical examination revealed the following figures per 100 cc: total protein, 2.0 to 3.19 g; albumin, 0.96 to 1.9 g; globulin, 0.6 to 0.8 g; fibrinogen, 0.2 to 0.41 g; non-protein nitrogen, 18 to 40 mg; sodium chloride, 700 to 750 mg; and calcium, 6.8 to 7.8 mg. The albumin: globulin ratio varied from 1.1:1 to 2.3:1 (Table I). It has been pointed out that group specific agglutinins are present in peritoneal and pleural transudates,[11-13] and that such substances are similar to those occurring in blood serum. Similarly, group specific substances are present in the lower animals, *e.g.*, in dogs.[14, 15] In view of these facts, the compatibility of the fluid with the blood of the prospective recipient was carefully determined. In each instance a cross-matching test was performed.

A series of 15 dogs, weighing from 7-10 kg, was used. Nembutal anesthesia was induced and shock was produced by graded hemorrhage. The criterion of shock was a systolic blood pressure

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[7] Amberson, W. R., and Höber, R., *J. Cell. Comp. Physiol.*, 1932, **2**, 201.

[8] Malinovskij, M. S., Smirnova, L. G., Boyarshinova, M. S., and Tarzanova, V. G., *Sovet. Khir.*, 1934, **7**, 179.

[9] Novikova, L. A., and Farberova, R. S., *Sovet. Khir.*, 1936, No. 11, 794.

[10] Yudin, S. S., *Lancet*, 1937, **2**, 361.

[11] Weil, P., and Isch-Wall, P., *Comp. Rend. Soc. de Biol.*, 1923, **88**, 173.

[12] Yosida, K. I., *Z. f. d. ges. exp. Med.*, 1928, **63**, 331.

[13] Schiff, F., *Uber die gruppenspezifischen Substanzen des menschlichen Körpers*, pp. 71, Fischer, Jena, 1931.

[14] von Dungern, E., and Hirschfeld, L., *Z. f. Immunitäts. u. exp. Therap.*, 1911, **8**, 526.

[15] Thomsen, O., and Kemp, T., *Z. f. Immunitäts. u. exp. Therap.*, 1930, **67**, 251.

TABLE I.
Composition of Human Ascitic Fluid.

No.	Condition	Kahn Reaction	N.P.N. mg per 100 cc	Fibrinogen g per 100 cc	Albumin g per 100 cc	Globulin g per 100 cc	A/G Ratio	Total Protein g per 100 cc	NaCl mg per 100 cc	Ca mg per 100 cc
7	Portal Cirrhosis	Neg.	22	.2	0.96	.84	1.1:1	2.0	750	7.8
10	,, ,,	,,	40	.41	1.9	.88	2.1:1	3.19	700	7
12	,, ,,	,,	18	.3	1.4	.6	2.3:1	2.3	750	6.8

TABLE II.
Influence of Ascitic Fluid in Hemorrhagic Shock.

Dog No.	Wt, kg	Calculated blood vol., cc	Amt of blood removed	Blood removed expressed as % of body wt	Amt of ascitic fluid, cc	Blood pressure mm Hg			Results
						Before hemorrhage	After hemorrhage	After ascitic fluid administered	
C-6	7.4	666	250	3.4	500	144	55	120	Survival
G-4	9.6	864	380	3.9	550	170	64	128	,,
B-5	8.2	738	350	4.2	520	135	50	110	,,

of 60 mm Hg or less. Kymographic tracings of the blood pressure were made in the usual manner from the carotid artery. Ascitic fluid warmed to 33C was allowed to flow into the femoral vein at a rate of 15 cc per minute. The infusion was commenced as soon as the blood pressure had maintained a constant level for a period of 3 to 5 minutes. Chemical examinations of the urine were made daily following each experiment for a period of 3 weeks. A control series of 10 normal animals was utilized.

In mild and moderately severe secondary shock, the infusion of human ascitic fluid was efficacious in raising and maintaining the systolic blood pressure well above the shock level. The elevation of the systolic blood pressure was gradual and usually reached a total of 50-60 mm Hg above the shock level. Not infrequently, there occurred a slight drop of blood pressure upon cessation of the infusion, but subsequently a return to the initial level took place. During the infusion, the respirations, which were rapid and shallow during the period of shock, became deeper and slower. It was found that the optimum amount of fluid varied with each animal, but did not exceed the calculated normal blood volume of the individual dog. The animals survived indefinitely, and revealed no immediate or delayed effects from the infusion. Examination of the urine showed a complete absence of albuminuria, glycosuria and haematuria. Apparently, the kidneys did not sustain any injurious effects from the ascitic fluid. Moreover, the consistent absence of albuminuria suggests that the proteins of the fluid are utilized by the animal. Representative data are presented in Table II. Injection of ascitic fluid into the control animals produced a rise of blood pressure of 15 to 30 mm Hg above the initial level, and a return to normal within twenty minutes.

These studies indicate that human ascitic fluid is of value as a substitute for whole blood. It possesses certain advantages: prolonged storage without preservatives does not affect its physiological availability in the organism; it is a fluid containing human proteins; it costs nothing; a constant source of supply is present in every hospital in patients suffering from various forms of chronic cardiac decompensation or from portal cirrhosis of the liver; it is sterile; its use obviates the dangers attending the use of proteinless infusions in secondary shock.[16]

Conclusion. Human ascitic fluid, when administered to group-compatible recipients, is capable of raising and maintaining the blood pressure in secondary shock.

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[16] Davis, H. A., *Arch. Surg.*, 1937, **35**, 461.

9897

A Practical Method for Preparation and Isolation of *l*-beta-Hydroxybutyric Acid.*

HARRY BLUNDEN. (Introduced by H. J. Deuel, Jr.)

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The increasing number of studies on the metabolism of ketone bodies has made a method for the preparation of pure *l*-beta-hydroxybutyric acid desirable. The easiest way to accomplish this is to synthesize it in the animal body. Although it may be obtained from the urine of fasting human subjects, it may also be readily produced in large amounts from the urine of fasted rats fed sodium butyrate according to the earlier procedures from this laboratory (0.237 g of butyric acid daily per 100 g rat in solution by stomach tube in divided doses morning and evening). When 85 female rats weighing 125-150 g were used, 58 g of *l*-beta-hydroxybutyric acid were excreted in a 4-day period of which 34 g were recovered as the pure Ca-Zn double salt. Although a similar procedure might be employed with a smaller number of dogs, our experience has been that rats are particularly well adapted because they can not regurgitate. The following method was employed in separating the acid in pure form.

To the pooled urines is added sufficient solid copper sulfate to obtain a concentration of 20%. To the resulting solution is added 0.5 g calcium hydroxide (in 10% aqueous suspension) for each gram of copper sulfate present. This amount of the base usually suffices to bring the pH slightly alkaline to litmus. If the pH is not alkaline to litmus after standing ½ hour more calcium hydroxide should be added. The mixture is then filtered by suction, working the precipitate with a spatula to a dry, hard cake, to express as much of the liquid as possible.

The filtrate is evaporated under reduced pressure to a syrup. At this point the precipitated salts may be filtered off. The resulting syrup is then acidified to litmus with 50% sulfuric acid. Caution must be exercised here to prevent the temperature of the syrup rising above 10°C, otherwise conversion to crotonic acid may occur. Then one full equivalent of 50% sulfuric acid is added to the syrup for each equivalent of *l*-beta-hydroxybutyric acid present as determined by assay according to the Van Slyke procedure.

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* This work was assisted by a grant from the Rockefeller Foundation.

Plaster of Paris is stirred into the acidified syrup in sufficient quantity to make a thick paste. As soon as the paste begins to set, one stirs it vigorously until a fairly coarse meal results. More plaster of Paris may be incorporated until the mixture is fairly dry.

The meal is then extracted with redistilled ether in a Soxhlet apparatus for 8 hours, after which the ether is evaporated off and the residue taken up with a minimum quantity of water.

The cloudy, slightly colored liquid is then shaken cold with a small amount of Norite and filtered; the resulting clear solution is divided into 2 equal volumes and neutralized by warming it with calcium carbonate and zinc carbonate respectively as outlined by Shaffer.[1] It is desirable to allow the 2 equal portions of the solution of beta-hydroxybutyric acid to stand for 12 hours in contact with an excess of the respective carbonates.

The excess carbonates are filtered off and the 2 solutions poured together. If more than 10% of the double salt of *l*-beta-hydroxybutyric acid is present, the calcium zinc double salt crystallizes out immediately and the addition of an equal volume of hot ethyl alcohol causes almost all of the remaining salt to crystallize out in 24 hours. The calcium zinc double salt may be purified by recrystallizing from water to which ethyl alcohol is added and finally from water.

The pure free *l*-beta-hydroxybutyric acid can now be recovered by acidifying a solution of the salt, setting with plaster of Paris and extracting with ether. After evaporation of the solvent the syrupy acid remains free from any contaminating substance.

After 3 recrystallizations from alcohol and one from water a preparation having the following constants was obtained:

	Found	Theoretical
$[\alpha]_{18°}^{D}$	—16.21°	—16.26°[1]
Ash ($CaO + ZnO$)	26.50	26.55%
Van Slyke assay	79.1% oxidation to Hg acetone precipitate	75.9% oxidation to Hg acetone precipitate

This method of preparation and solution has been employed several times successfully by the author and independently by another investigator in this laboratory (B. Bobbitt) who obtained a higher percentage recovery than is reported here.

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[1] Shaffer, P. A., and Marriott, W. M., *J. Biol. Chem.*, 1913-14, **16**, 265.

9898 P

Movement of Water Against a Gradient in Models.

W. J. V. OSTERHOUT AND J. W. MURRAY.

From the Laboratories of the Rockefeller Institute for Medical Research.

In certain models employed to imitate living cells the behavior of water appears to be the opposite of what is expected. If it acts similarly *in vivo* it is not hard to understand why the living cell often fails to behave as a perfect osmometer.

In these models water moves through liquid layers against a gradient, passing from a region where its activity is low to one where its activity is high. This depends upon the fact that certain substances traversing these layers appear to carry water with them.

The simplest form of model is made as follows: In a U-tube (Fig. 1) we place guaiacol* (B) to imitate the action of the protoplasm.[1] Resting on the guaiacol (which forms a separate phase in contact with water) at the left is an aqueous solution (A) representing the external solution, and at the right an aqueous solution (C) representing the cell sap.

We may begin by putting in B some guaiacol which has been shaken with distilled water until equilibrium has been attained: in A and C we place equal amounts of the water which has been shaken with guaiacol. The system is therefore in equilibrium throughout and no movement of water occurs.

When we lower the activity of the water in A by adding trichloroacetic acid we might expect water to move from C to A, but just the opposite occurs. Acid and water move from A to B and from B to C. The higher the concentration of acid in A the greater the movement of water into C.

Evidently the water moves from A to C because acid does so and hence it would appear as though the acid carries water with it. This could be easily understood if the acid formed a hydrate (or a series of hydrates) so that it could not pass into C without carrying water with it. But it is not necessary to make such an assumption, for it is easily shown by shaking A and B together that acid increases the solubility of water in B (a "salting in" process). But when the acid moves from B to C the solubility of water in B decreases and in consequence water goes from B to C.

As the freezing point of A is lower than that of C it is evident

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* O-methoxyphenol.

[1] *Cf.* Osterhout, W. J. V., and Stanley, W. M., *J. Gen. Physiol.*, 1931-32, **15**, 667.

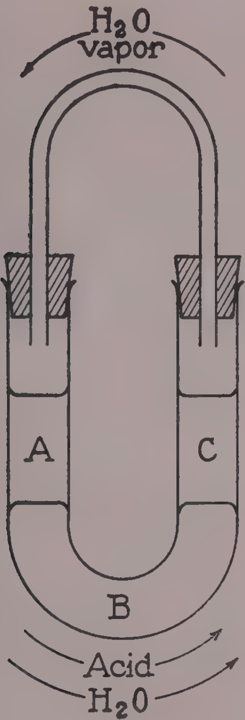

FIG. 1.

A contains an aqueous solution of trichloroacetic acid. B contains guaiacol. C is water saturated with guaiacol at the start. Acid and water move from A through B to C. In the air above the solutions water vapor moves from C to A.

that water moves from A where the activity of water is low to C where its activity is high. The movement of water vapor through the air above the solutions is from C to A. This apparent "perpetual motion" can go on for months, ceasing only when A and C become identical in volume and in composition.

The increase of water in C may amount to 40% or more. In view of this result we thought it desirable to test it in another way, namely by shaking A with B and then shaking B with C (in both cases the shaking was continued until equilibrium was attained). The result shows an increase of water in C which may amount to more than 400%.

It seems possible that any substance which causes guaiacol to take up water may act to some extent like trichloroacetic acid in these experiments. This has been found to be true of acetone, but the results are much less striking.

The behavior of water here described seems to be a different phenomenon from "anomalous osmosis" through solid membranes which appears to require the presence of pores and of electrolytes.[2]

The gain in water in C is obviously only temporary. If left to itself the system would undoubtedly reach equilibrium with A and C identical in composition and in volume.

Since in certain respects guaiacol acts like some protoplasmic surfaces[1] it seems possible that similar phenomena may occur in living cells. If so these results have an obvious bearing on the movement of water in the organism and on methods of studying permeability. It becomes necessary to know to what extent a substance entering or leaving the cell carries water with it in the manner here indicated.

9899

"Acid" Phosphatase Activity of the Serum of Normal Human Subjects.

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Though the optimum for phosphatase activity of normal serum is in a distinctly alkaline range, pH-activity curves indicate slight but definite activity on the acid side of neutrality.[1] It has not been made clear whether these curves reflect the operation of a single enzyme over an unusually wide range in pH, or whether they are really composite curves representing the activities of 2 or more enzymes. Data to be presented suggest that the phosphatase activity of normal serum in the acid range is due largely to the presence of minute amounts of one or more phosphatases falling within the group classified by Folley and Kay[2, 3] as phosphomonoesterase A_2.

The method of King and Armstrong[4] proved adaptable to the estimation of "acid" serum phosphatase activity under conditions of hydrolysis most likely to approximate initial reaction velocities. The buffer-substrate employed was M/200 disodium monophenylphosphate in Sørensen's M/10 citrate-HCl buffer adjusted to pH 4.8. After addition of serum, the pH of the reaction mixture at room temperature was 4.85 (glass electrode measurements) and therefore approximately 4.9 at the temperature of hydrolysis, 37°C. The optimum time of hydrolysis for satisfactory colorimetric readings was found to be 3-5 hours. This period may be shortened by using higher concentrations of substrate or by making photometric readings. However, time-activity curves indicated that after hydrolysis for even 5 hours, the deviation from linearity, while significant, was not sufficient to offset advantages of the method when applied to sera in certain diseases.[5]

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[2] *Cf.* Söllner, K., *Kolloid-Z.*, 1933, **62**, 31.

[1] Roche, J., *Biochem. J.*, 1931, **25**, 1724.

[2] Folley, S. J., and Kay, H. D., *Ergebn. d. Enzymforsch.*, 1936, **5**, 159.

[3] Folley, S. J., and Kay, H. D., *Tabulæ biol. period.*, 1937, **6**, 268.

[4] King, E. J., and Armstrong, A. R., *Canad. M. A. J.*, 1934, **31**, 376.

The usual precautions against hemolysis were taken. Since the "acid" phosphatase activity of many sera falls after standing several days in the refrigerator, fresh sera were used throughout. The results are expressed in units per 100 cc serum, a unit being defined as that degree of phosphatase activity which at pH 4.9 and 37°C will liberate from the specified buffer-substrate solution 1 mg of phenol in 1 hour.

The phosphatase activity of 23 normal sera determined at pH 4.9 by this method ranged from 0.6 to 2.0 units per 100 cc serum (*vide* Table I for 12 representative values). The results obtained are due to enzymatic, not acid hydrolysis, as shown by the absence of scission in control experiments in which substrate-buffer mixtures were maintained at 37°C for 5 hours without the addition of serum. That the enzyme operating at pH 4.9 is not "alkaline" serum phosphatase, however, is suggested by the lack of correlation between the "alkaline" and "acid" activities of normal sera, notably in children (Table I). Sera in diseases presenting marked elevations in "alkaline" phosphatase activity show no proportionate rise in "acid" phosphatase activity (Table I).* Further, unlike "alkaline" serum phosphatase, the "acid" phosphatase activity of serum is markedly inhibited by M/100 NaF and is not accelerated by Mg ion (Table

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[5] Gutman, A. B., and Gutman, E. B., *J. Clin. Invest.*, in press.

* If a single enzyme (*i.e.*, "alkaline" serum phosphatase) were operative and inactivated only in part at pH 4.9, values for "acid" serum phosphatase would be correspondingly lower than those at pH 9.0 but nevertheless proportional to them,[6] as occurs, for example, when hydrolysis is conducted at pH 7.6 (Kay's method). If a single enzyme were operative and wholly inactivated at pH 4.9, the phosphatase activity at pH 4.9 would, of course, be zero. It was found that after incubating for one hour at pH 4.9 (using M/10 citrate buffer), the phosphatase activity of serum brought back to pH 9.0 by addition of NaOH was not more than 25% and usually less than 10% of the value of untreated serum.

[6] Martland, M., and Robison, R., *Biochem. J.*, 1927, **21**, 665.

TABLE I.

"Acid" and "Alkaline" Phosphatase Activity of Serum.

(Substrate = M/200 disodium monophenylphosphate; buffer = M/10 Sørensen's citrate-HCl (pH 4.9); temperature = 37°C)
= M/20 sodium veronal (pH 9.0)

No.	Subject	Age	Sex	Phosphatase activity (mg phenol liberated in 1 hr, per 100 cc serum)	
				pH 4.9	pH 9.0
1	Normal adult	24	♂	2.0	8.3
2	,, ,,	31	♂	1.4	9.0
3	,, ,,	49	♂	1.3	9.0
4	,, ,,	28	♂	1.3	17.5
5	,, ,,	29	♂	1.1	13.7
6	,, ,,	25	♂	1.1	14.4
7	,, ,,	28	♀	1.0	6.8
8	,, ,,	23	♂	0.9	14.1
9	Normal adult	25	♂	0.8	10.3
10	,, ,,	25	♂	0.6	8.6
11	,, child	4.5	♂	2.0	33.4
12	,, ,,	4	♂	1.8	42.4
13	Advanced Paget's disease	63	♀	5.0	386.
14	,, ,, ,,	55	♂	3.4	583.
15	Hyperparathyroidism	50	♀	2.3	134.
16	Obstructive jaundice	41	♀	1.7	143.

TABLE II.

Representative Experiments Illustrating "Acid" Phosphatase Activity of Normal Serum Under Varying Conditions of Hydrolysis.

Exp. No.	Substrate	Buffer Citrate-HCl	pH	Agent added	Time of hydrolysis hr	Phenol liberated, mg per 100 cc serum	Comment
1	M/200 phenylphosphate	M/10	4.9	0	3	4.2	Control
	M/200 ,,	M/10	4.9	M/100 NaF	3	1.4	Marked inhibition of "acid" phosphatase by fluoride
2	M/200 ,,	M/10	4.9	0	3	4.0	Control
	M/200 ,,	M/10	4.9	M/50 $MgCl_2$	3	3.5	No activation of "acid" phosphatase by Mg ion
3	M/40 α glycerophosphate	M/25	4.9	0	5	2.2	α glycerophosphate is not more rapidly hydrolyzed than β glycerophosphate
	M/60 β ,,	M/25	4.9	0	5	2.6	
4	M/200 phenylphosphate	M/10	4.9	0	3	3.9	Control
	M/200 ,,	M/10	4.9	M/1 propyl alcohol	3	3.9	No inhibition by alcohols

II). The "acid" phosphatase activity of serum, moreover, is less stable on standing than is "alkaline" serum phosphatase and is more slowly inactivated by heating to 60°C.

The "acid" phosphatase of normal serum was found to have a broad range of optimum activity from about pH 4.0 to pH 6.5. This, together with the characteristics summarized in Table II suggest that the enzyme concerned is best classified, for the present, as a phosphomonoesterase A_2. The available data indicate that "acid" serum phosphatase is not identical with erythrocyte phosphatase[1] or with the "acid" phosphatase found in normal prostate tissue[7] and in the skeletal metastases[8] and serum[5] of patients with disseminated prostate carcinoma. As regards phosphatases occurring in the liver and spleen,[9, 10] however, no serious inconsistencies are apparent at this time. These organs may be a source of the "acid" phosphatase content of the serum.

Conclusion. In addition to "alkaline" phosphatase, blood serum contains minute amounts of one or more "acid" phosphatases of the type classified by Folley and Kay as phosphomonoesterase A_2.

9900

A Micro Precipitin-Technic for Classifying Hemolytic Streptococci, and Improved Methods for Producing Antisera.

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The increasing practice of grouping hemolytic streptococci serologically by the use of the precipitin-reaction has stimulated us to devise a microtechnic whereby the accuracy of the original method is retained with a great saving of material. Potent sera are essential; and for the maintenance of a steady supply, it has been found advantageous to keep on hand rabbits which are repeatedly immunized and bled, with rest periods between the courses of treatment.

It is important to select suitable strains for immunization. The two methods of immunization employed lead to the production of precipitins for both the group-specific carbohydrate (C) and the type-specific substances, which are proteins (M) for the types within Group A and probably polysaccharides (S) for the types within most of the other groups. Consequently, those strains that have lost their type-specific substances—either spontaneously or induced by serial subcultures in type-specific serum or by selection of non-type-specific colonial forms (glossy for Group A, rough for other groups)—are the best antigens when the formation of group-specific precipitins is the object. Such strains have been prepared for several of the groups. Group D has proved especially difficult.

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[7] Kutscher, W., and Wörner, A., *Z. f. physiol. Chem.*, 1936, **239**, 109.

[8] Gutman, E. B., Sproul, E. E., and Gutman, A. B., *Am. J. Cancer*, 1936, **28**, 485.

[9] Davies, D. R., *Biochem. J.*, 1934, **28**, 529.

[10] Bamann, E., and Riedel, E., *Z. f. physiol. Chem.*, 1934, **229**, 125.

When strains devoid of type-specific substance are not available, a strain of the same *group*, but of a different serological *type* from that used in immunization, is employed in preparing the extract for the precipitin-test. This eliminates a possible complicating type-specific antigen-antibody reaction. If the homologous strain, containing type-specific antigen, were used in this test, the extract would contain homologous specific substances for both *group* and *type*, and it would be impossible to distinguish between type- and group-specific precipitins, one or both of which might be present. These precautions are necessary in testing sera which are being prepared, although they are not important in the identification of new strains once suitable group-antisera have been obtained.

Method of Immunization for Preparing Group-Antisera. In Group A, heat-killed cultures have been found at least as satisfactory antigens as formalin-killed bacteria for preparing antisera. Eighteen-hour broth cultures are heated at 56°C for one hour. The first week the rabbits are given 5 daily intravenous injections of 1 cc of whole broth culture followed by a rest period during the second week. In the third week, five 2 cc doses are given, and in the fifth week, five 4 cc doses. The larger doses are given in volumes of 1 to 2 cc prepared by centrifuging the culture and resuspending the bacterial sediment in broth or saline. If further immunization is indicated, it is better to repeat the 4 cc doses of vaccine rather than to increase the amount or to give living culture. Five days after the last injection of the second series, a test bleeding is made. If a sufficiently high precipitin-titer is found, 50 cc of blood are taken from the ear vein into a centrifuge tube lined with a thin layer of sterile vaseline, which, by allowing the clot to separate from the tube spontaneously, favors a large yield of colorless, clear serum. Immunization is continued according to schedule, and 50 cc of blood are obtained every 2 weeks until the antibody-titer begins to fall. The animal is then rested for 2 months or longer. When more serum is needed, injections are begun again, starting with 1 cc doses as before, with the precaution of giving the first few intraäbdominally or subcutaneously to avoid death of the animal from shock. Animals which have rested from 2 to 12 months usually respond satisfactorily after one series of injections, although 2 or more series may occasionally be required. Furthermore, rabbits that were originally unsatisfactory in their response may on secondary injection produce high-titered antisera. This procedure has proved especially helpful with Groups G and H. It is advisable to keep 2 rabbits on hand for each group and replace them when necessary on account of age or intercurrent infection, such as snuffles. We have kept numerous animals immunized in this way for 3 or 4 years. A typical rabbit producing Group A serum has been under immunization for 3½ years with 6 long rest periods, and has yielded 500 cc of high titered serum, half of which was obtained during the first year.

For preparing antisera to all groups except Group A, formalin-killed cultures have been found much better than heat-killed. The sediment from an 18-hour broth culture is suspended in 0.2% formalin in saline in one-twentieth the volume of the original culture. This is stored in a tightly stoppered container in the icebox. Periods of one to 4 or 5 days are required to kill the streptococci, the time varying according to the serological group of the strain. Immediately before use, a 1:20 dilution of the formalized cell suspension is prepared. Six intravenous injections of 1 cc each are given daily in alternate weeks. Test bleedings are made after the second series, and the main bleedings and rest periods are arranged on the same schedule as that used for Group A animals.

Slight cross-reactions between certain groups have been reported by numerous observers, most often in Group C antisera. These cross-reactions are usually observed only after the tubes have stood in the icebox overnight. When there is a sufficiently high group-antibody content, the cross-reactions are insignificant. When, on the other hand, the homologous group-reaction is weak, these cross-reactions with heterologous groups become relatively more significant and lead to confusion. The cross-reacting capacity of potent group-antiserum may be eliminated by absorbing the serum with heat-killed streptococci from a strain of another serological group, but absorption is not usually found necessary. However, 5 cc of serum can be satisfactorily absorbed with 1 cc of packed bacteria of a heterologous group if the procedure seems advisable.

Preparation of Extracts. Extracts are prepared as previously described.[1] The bacterial sediment from 250 cc of broth culture is suspended in 10 cc of N/20 HCl in 0.85% NaCl. The suspension is sufficiently acid to turn Congo Red paper blue. The tube is immersed in a boiling-water bath for 10 minutes, cooled under running water, and centrifuged. The supernatant fluid is removed and neutralized and the resulting inactive precipitate is discarded after centrifugation. The final supernatant fluid is used in the precipitin-reaction.

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[1] **Lancefield, R. C., *J. Exp. Med.*, 1933, 57, 571.**

For the micromethod smaller amounts in the same proportion are used. The bacteria centrifuged from 50 cc of a luxuriant growth of streptococci in plain or dextrose broth (or in blood-neopeptone broth for the fastidious organisms in Groups E, F, and G) are extracted in 2 cc volume. More dilute extracts lessen the speed and sensitivity of the reaction especially when the "ring" test in small volumes is used.

Microtechnic. An innovation has been introduced in performing the precipitin-test in a small volume. We have previously hesitated to use micro-methods because of possible misinterpretations due to prozonal phenomena, or to cross-reactions which, in small volumes, might appear to be of the same order of magnitude as the major group-reaction. However, with high-titered sera and sufficiently concentrated extract, we find that a ring-test in a single dilution in a tube of small caliber eliminates the danger of negative reactions due to the prozone by giving mixtures of varying concentrations at the junction of extract and serum. The correctness of the results is further safeguarded by applying the dilution-method to doubtful cases.

A small conical tube is prepared by slightly drawing 7 mm glass tubing in a flame. The drawn tubing is cut in the center to make 2 tubes. The narrow end is sealed into a knob to prevent the tube from slipping through the rack. The 7 mm diameter is retained at the open end to facilitate pipetting, and the lower end has a bore of about 3 mm, holding 0.1 cc in a column 8 to 10 mm high. Good results have been obtained by putting 0.05 cc of the extract into the tube first, and then 0.05 cc of serum. Since the serum is heavier, it sinks below the saline extract and forms a layer with about the right amount of mixing. With the usual technic employed in ring-tests of placing the serum in the tube first, too narrow a ring is formed due to insufficient mixing in a tube of such small caliber. If, due to an air bubble, the fluid fails to lodge at the bottom of the tube, no attempt is made to shake it down. The result can easily be read at any level, and shaking is apt to interfere with the formation of a good plane of junction. Positive reactions are usually obvious at once, and can be safely recorded after ½ hour at room temperature. In order to save serum, exceptionally potent antisera may be diluted, but not beyond the point where an immediate heavy ring-reaction is obtained with extracts of the homologous group.

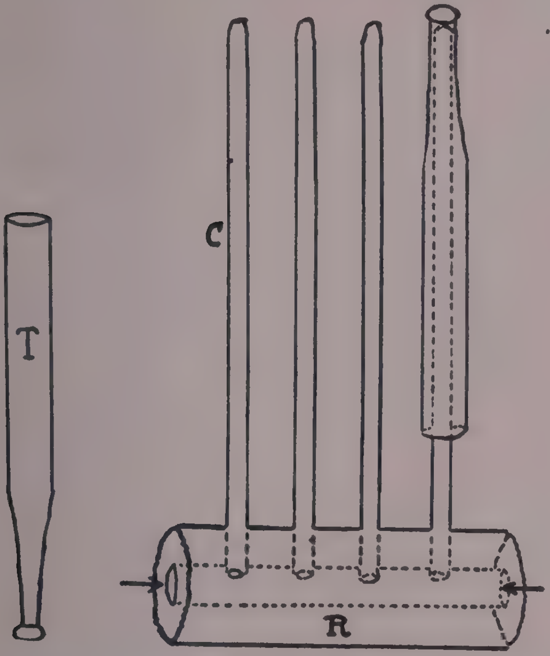

FIG. 1.

Precipitin-tube for microtechnic and apparatus for washing tubes. T. Precipitin-tube; R. Section of rubber pressure-tubing; C. Copper outlet-tube. Arrows indicate water-intake.

In case of doubt, the dilution-method with large volumes is used, or a comparable dilution-method can be employed with the microtechnic in the following manner: 0.05 cc each of undiluted extract and of 1:4 and 1:16 dilutions of extract are pipetted into 3 small tubes, and 0.05 cc of serum is added to each. The small tubes are observed for ring formation, as usual, at the end of a half hour. The tubes are then shaken, incubated at 37°C for 2 hours and placed in the icebox overnight before final readings are made.

The small tubes must be carefully cleaned. A convenient washing apparatus consists of a length of pressure-tubing into which holes have been punched to contain a row of outlets made of ⅛ in. copper tubing about 4 in. long. Running water enters the pressure-tubing at both ends. The small test tubes are first inverted over these outlets to wash out the contents. They are then immersed in bichromate cleaning solution, washed with running tap water on the apparatus described, and finally rinsed in distilled water and dried for use.

We have recently reëxamined 175 cultures of hemolytic streptococci, which had previously been grouped. The micromethod agreed completely with earlier results and there was no uncertainty in reading the reaction. It seems, therefore, that the method of using a ring-test with small amounts of material is feasible if one observes the safeguards of employing concentrated extracts and potent antisera, absorbed when necessary. In case of doubt the results can always be confirmed by the dilution-method.

The discussion so far has dealt with the classification of hemolytic streptococci into broad serological *groups*. The methods described are, however, also applicable to the differentiation of serological *types* within these groups. Further details of the micromethod for the precipitin-test as applied to the classification into types of members of Group A will be given later.

It should be noted that certain non-hemolytic strains of streptococci may belong to some of the serological groups originally differentiated among the hemolytic streptococci. Stableforth was the first to show that some non-hemolytic streptococci isolated from bovine sources are members of Group B.[2] Sherman has made the same observation with reference to Group D.[3] We also have obtained occasional non-hemolytic members of Groups B, C, D, and G, some of which are serologically identical with known immunological types within the groups represented.[4]

Summary. Methods are described for producing high-titered group-specific antisera for classifying hemolytic streptococci into groups. A description is also given of a reliable micro-precipitin technic which can be used with very small amounts of bacterial extract and antiserum. Attention is called to the occurrence of some non-hemolytic members of Groups B, C, D, and G.

Since this communication was submitted for publication, Fuller has reported a method of extracting streptococci with formamide.[5]

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[2] Stableforth, A. W., *J. Comp. Path. and Therap.*, 1932, **45**, 185; *J. Path. and Bact.*, 1937, **45**, 279.

[3] Sherman, J. M., *J. Bact.*, 1938, **35**, 81.

[4] Lancefield, R. C., *J. Exp. Med.*, 1934, **59**, 459.

[5] Fuller, A. T., *Brit. J. Exp. Path.*, 1938, **19**, 130.

9901 P

Explanation for the Cyanosis of Sulphanilamide Therapy.*

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The extraordinary cyanosis shown by patients during sulphanilamide therapy has received no explanation.

Marshall,[1] King,[2] and others have demonstrated that the respiratory function of the hemoglobin is unaltered. Methemoglobin and sulphemoglobin have been found in some cases but in amounts far too small to explain the intense cyanosis. The plasma of cyanotic patients appears normal. Whole blood, whether arterial or venous, has a chocolate brownish tinge. Though such blood when aerated gains oxygen in normal amounts it does not become bright red but remains dark. This led us to seek for a colored derivative of sulphanilamide in the erythrocytes.

After various unsuccessful attempts we were led by the known helio-sensitivity of sulphanilamide-treated patients to try the effect of ultraviolet light. This enabled us to reproduce the phenomenon *in vitro* and to offer an explanation for the cyanosis.

We found that dilute solutions of sulphanilamide on very brief exposure to ultraviolet light develop a strong violet color. The nature of the chemical change is so far unknown.

When red cells are added to such solutions they at once adsorb the violet substance from the supernatant fluid and assume the color shown by the red cells of patients under sulphanilamide therapy. Plasma added to the violet solutions causes the color to disappear.

The violet substance is only the first of several colors produced by irradiation of sulphanilamide. It is developed only by irradiation of short duration, one half to one or at most 3 minutes. When irradiation is continued for longer periods the color rapidly changes to yellow, then brownish.

When the violet solutions are allowed to stand a slow spontaneous change occurs. In an hour the violet fades markedly and a more permanent pinkish purple of less intensity appears.

The violet color is only produced by irradiation of dilute sulph-

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* Work done under tenure of the Theodore Escherisch Fellowship with assistance of the Emanuel Libman Fellowship Fund.

[1] Marshall, E. K., Jr., and Walzl, E. M., *Bull. J. Hop. Hos.*, 1937, **61**, 140.

[2] King, F. H., and Leslie, A., *J. A. M. A.*, in publication.

anilamide solutions—of a concentration in fact approximating that in the blood of treated patients—namely, one to 15 mg per 100 cc. When more concentrated solutions are used the yellow color dominates from the start (Table I). In numerous repetitions of these experiments it was found that the intensity of the colors varied with the depth of the fluid layer irradiated.

TABLE I.
5 cc of Each Solution in 9 cm Petri Dish Irradiated for 60 seconds with 110 V.D.C. Hanovia Lamp. Distance 40 cm.

Mg sulphanilamide per 100 cc	Color developed
0.8	very faint violet
1.6	faint violet
5.0	violet +++
8.0	” +++
10.0	” ++++
16.0	blue, brownish red
32.0	dirty yellow
48.0	yellow ++
64.0	” +++
80.0	” +++

When dilute suspensions (2%) of red blood cells in sulphanilamide saline solution are irradiated they undergo the color change but do not absorb the violet color from the supernatant solution as do freshly added red blood cells. Possibly they are already saturated with the color substance. They show slight hemolysis as do all irradiated red cells.

When quantitative determinations by Marshall's method[3] are made of the sulphanilamide in the violet solutions they are found to have lost 30 to 60% of their sulphanilamide (Table II). Repeated series of irradiations showed that the percent of sulphanilamide changed depended on several factors such as duration of irradiation and the depth of the fluid layer. As Marshall's method depends on diazotization it is probable that the color change involves the NH_2 group. Pointing in the same direction is the increased acidity of the solutions after radiation (electrometric pH determination). Beyond this we can as yet offer no chemical explanation. The possibility of oxidation was considered: sulphanilamide mixed with peroxide of hydrogen shows no change, but on irradiation develops a reddish yellow color. Irradiation of some chemically related simple compounds such as aniline and sulphanilic acid, develops no color.

Whether any of the colored derivatives of sulphanilamide produced in the body will by its superior bactericidal power explain the

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[3] Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263.

TABLE II.
Concentration of Sulphanilamide in Aliquot Portions, Before and After Production of Violet Substance by Irradiation.

Before radiation, mg per 100 cc	After radiation, mg per 100 cc	% decrease
6.3	2.7	53
6.3	3.4	46
7.0	3.0	57
7.0	3.0	57
9.0	4.5	50
9.8	7.0	29
11.0	6.4	42
12.2	6.1	50

chemotherapeutic effect (insufficiently accounted for by the very feeble bactericidal power of the drug itself) remains to be ascertained.

In offering this phenomenon as the explanation of the cyanosis during sulphanilamide administration we do not suggest that light is the causative factor *in vivo*. It would seem that a transformation so easily produced by light *in vitro* might well be brought about by some of the rapid and powerful metabolic changes constantly occurring in the tissues.

9902

Skin Absorption of Dihydroxyestrin in Humans.

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The present investigation was undertaken to determine whether estrogenic hormone can be absorbed through the human skin. Absorption was estimated on the basis of the application to the human female of the Allen-Doisy test. Papanicolaou and Shorr[1] have demonstrated that the human vaginal secretions after the menopause or castration exhibit certain striking cytological features, characterized chiefly by the presence of small, round or oval epithelial cells ("deep cells") with rather large, well-staining nuclei and associated with a varying number of leucocytes. These investigators have shown that following administration of adequate amounts of estrogenic hormone the leucocytes and "deep cells" disappear and are supplanted by large, squamous epithelial cells, characteristic of the follicular phase of normally menstruating women. In the present study an attempt was made to determine whether similar vaginal smear changes could be produced in human females by means of skin inunctions with an estrogenic hormone.

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[1] Papanicolaou, G. N., and Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 585.

A group of women were chosen who were considered, on clinical grounds, to have either marked ovarian deficiency (post-menopause or X-ray castration) or no ovarian function at all (bilateral oöphorectomy). Of these, 14 were selected whose vaginal smears during a period of observation of 2 weeks were found to be constantly "negative" (Reaction I or O),[2] indicating absence of estrogenic activity. The vaginal mucous membrane changes of this group of women, as revealed in the smears, constitute sensitive test objects for estrogenic substances.

Seven groups of 2 cases each were put on a lanolin ointment containing concentrations of dihydroxyestrin* varying from 500 to 25,000 R.U. per ounce. The ointment was applied to the face, neck, and chest nightly, one hour before retiring. By varying the concentration of the hormone in the ointment, as well as the quantity of ointment used with each inunction, the amount of dihydroxyestrin applied to the skin was varied from 500 R.U. to 100,000 R.U. per week. It is obvious that, since it would be impossible to prevent loss of some of the ointment on bed clothes, all calculations would have to be only approximate. Vaginal smears were taken at weekly intervals, prepared and classified as previously described.[2]

At the end of 1 week, the smears of the 2 patients who had used 100,000 R.U. of dihydroxyestrin began to show early signs of cell changes, consisting of a diminution in the number of leucocytes and an increase in the size and number of the epithelial cells (Reaction II). During the following week, with continued inunctions, the leucocytes and "deep cells" grew steadily less in number and were supplanted by increasingly larger numbers of squamous epithelial cells (Reaction III). At the end of 3 weeks, the smears consisted entirely of large squamous epithelial cells with small, deeply-staining nuclei (Reaction IV). A total of 300,000 R.U. of the estrogen was applied before this effect was obtained.

The group using 40,000 R.U. per week began to show early estrogen effects in the smear (Reaction II) at the end of 2 weeks and a full estrogen effect (Reaction IV) at the end of 4 weeks when

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[2] Salmon, U. J., and Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 612.

* For the dihydroxyestrin ointment used in this study, I am indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

160,000 R.U. had been used. The smears of the 2 cases using 10,000 R.U. per week showed only an incomplete estrogen effect at the end of 8 weeks, when 80,000 R.U. had been used. Although the squamous epithelial cell was the predominating type of cell in the smears of these cases a few "deep cells" and leucocytes were still present at the end of 8 weeks.

The 6 cases using 1,000 to 4,000 R.U. per week did not show any significant effect until after the 6th week. The changes in the smears were slight, consisting of a relative diminution in the number of leucocytes and "deep cells" and the appearance of scattered, larger epithelial cells. Although these patients kept on using the same amount of ointment for 6 more weeks, there was comparatively little further progress in the smears, leucocytes and "deep cells" being constantly present, though somewhat reduced in number. The smears of the 2 women using 500 R.U. per week showed no significant changes at the end of 12 weeks. Apparently these patients were not absorbing enough of the dihydroxyestrin to produce an estrogenic effect on the vaginal mucosa.

Of the 14 cases treated, a full estrogen effect (Reaction IV) was produced in 4; an incomplete effect (Reaction III) in 2; a slight effect (Reaction II) in 6, and no reaction in 2 cases. A full estrogen effect (Reaction IV) in these cases was produced with total doses of dihydroxyestrin varying from 160,000 to 300,000 R.U. The time elapsed before the full estrogenic effect appeared in the smear varied from 3 to 4 weeks. The quickest response was obtained in 3 weeks in a woman who used 12 ounces of an ointment containing 25,000 R.U. per ounce, representing a total skin application of 300,000 R.U. in 3 weeks. Beginning estrogen effects were noted as early as 1 week after the institution of inunctions when the concentrated preparation was used (25,000 R.U. per ounce) and as late as the 6th week with the weaker ointments (1,000 to 4,000 R.U. per ounce). The vaginal smears began to show signs of regression (manifested by the appearance of "deep cells" and leucocytes) within 2 weeks after the discontinuation of the inunctions.

It seems from these experiments that in order to produce a full estrogen effect more than 10,000 R.U. of dihydroxyestrin must be applied to the skin per week; when less than this amount is applied only a partial effect is produced in the smears, even though the inunctions are continued for 12 weeks.

It is interesting to note that 6 of the patients volunteered the information that they experienced considerable relief of the pruritus vulvae and vaginal discomfort of which they had formerly complained. This symptomatic improvement coincided with the appearance of an estrogenic effect in the vaginal smear. The skin at the site of inunctions, even in the cases using the most concentrated ointments, exhibited no signs of irritation.

Since the completion of this investigation, the attention of the writer was drawn to a publication by Loeser[3] in which he reports the use of dihydroxyestrin benzoate inunctions in women, using changes in the endometrium and clinical symptoms as an index of absorption. In 5 cases in which endometrial studies were done, he reported no significant changes in the endometrium following inunctions with a total of more than one gram (approximately 6,500,000 R.U.) of the hormone in each case. However, on the basis of clinical symptoms (swelling of the breasts, abdominal cramps and vaginal discharge) which appeared after the inunctions, Loeser concludes that the dihydroxyestrin benzoate is absorbed through the skin. It is worthy of note that in none of the cases reported in the present communication were any of the symptoms described by Loeser observed. The systemic symptoms noted by Loeser are probably attributable to the much larger doses of hormone which were used in his cases.

It appears from this study that dihydroxyestrin is absorbed through the skin of the human female and, when applied in sufficient concentration, exerts an estrogenic effect upon the epithelium of the vaginal mucous membrane. This is shown by the transformation of the vaginal smear, following inunctions with adequate amounts of an ointment containing dihydroxyestrin, from the "negative" phase, in cases of advanced estrogen deficiency, to a "positive" phase characteristic of normal ovarian activity.

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[3] Loeser, A. A., *J. Obst. and Gynec., Br. Emp.*, 1937, **44**, 710.

9903 P

Transmission of Mouse Sarcoma with Small Numbers of Counted Cells.*†

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Several of the current theories of the etiology of cancer[1] postulate the presence of malignant cells in apparently normal individuals. It is assumed that such isolated cells are incapable of producing a neoplasm unless they attain, in a small region, a large enough number to overcome resistance. This opinion is based in part on transmission experiments with carcinoma and sarcoma of laboratory animals. The smallest number of cells required for the transplantation of these neoplasms is estimated by different workers as between 12,500 and 1,000,000.[2]

Successful inoculations with tissue cultures containing a small, though uncounted, number of malignant cells led Dr. Warren H. Lewis[3] to believe that a few malignant cells might under favorable conditions suffice for the transmission of sarcoma. Our quantitative studies with transmissible leukemia indicate that a single malignant cell isolated in a microdroplet with the micropipette, prior to animal inoculation, may transmit the disease.[4] We have found it possible to use essentially the same technic in transmitting mouse sarcoma with counted numbers of cells. The tumor is derived from a sarcoma produced by subcutaneous injections of 1-2 benzpyrene. The following experiments indicate that this strain of sarcoma can be readily transmitted with from 50 to 100 cells.

Technic. The suspension of tumor cells was prepared in a manner similar to that of malignant lymphoid cells with the difference that the tumor fragments were washed with Tyrode solution to remove most of the blood that they contained. The washed particles were then cut up in a 10% solution of normal mouse serum in Tyrode solution. The tumor particles were allowed to settle out

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*This investigation has been supported by the International Cancer Research Foundation.

† These studies were made with the able technical assistance of Miss Ann Sonnentheil, Mrs. Fannie B. Parsons, and Mr. Charles Breedis.

[1] *Cf.* Fischer, A., *Am. J. Cancer*, 1937, **31**, 1.

[2] De Gaëtani, G. F., and Blottner, E., *Z. f. Krebsf.*, 1936, **44**, 108.

[3] Lewis, Warren H., personal communication to J. Furth.

[4] Furth, J., and Kahn, M. C., *Am. J. Cancer*, 1937, **31**, 276.

and the supernatant fluid containing free cells and small clumps of cells was pipetted off. These were subsequently isolated in microdroplets and counted prior to injection. Of the 12 successfully inoculated mice, 7 received a counted number of cells in each site injected; but in each of 2 mice the suspension containing a counted number of cells was divided equally and injected into 2 sites; and in each of 3 mice the suspension was divided equally and injected into 4 sites. The cell suspension used for inoculation contained mostly single cells and occasional small clumps of cells. Clumps of cells were injected only when the number of cells in them could be determined. The number of cells in an injected clump never exceeded 12. All cells that might possibly have been tumor cells were counted. Neutral red and trypan blue preparations indicated that most cells were alive.

The results of inoculation are shown in Table I.

TABLE I.
Number of Mice Successfully Injected with Counted Sarcoma Cells.*

Lot No.	No. of cells injected	No. of mice injected	No. of sites injected	No. of successful injections	No. of mice successfully injected
1	50 to 100	7	28	5	4
2	60 to 290	6	24	0	0
3	50 to 100	14	26	4	4
4	50 to 100	7	28	9	4
5	39 to 62	7	28	1	1
6	10 to 43	6	24	0	0
	Total	47	158	19	13

*All mice were injected with an exact number of cells. For instance, mouse No. 1 received 64 cells; mouse 2, 73 cells, etc.

Most of the unsuccessfully injected mice are still alive and it is possible that some of them will develop sarcoma at the site of injection, but these retarded tumors would in no way alter our conclusions. In one experiment 4 mice that failed to develop sarcoma received 450 r of X-rays, both groins where the inoculations had been made 3 months previously being shielded, but the mice remained free from growths. In another experiment, 3 of the 6 mice injected received 400 r of X-rays before inoculation, but none of these mice developed tumors. These experiments were undertaken to promote the growth of malignant cells should they remain dormant as suggested by Fischer,[1] but there is no evidence in our experiments in favor of this possibility.

Summary. Sarcoma of mice can be transmitted with approximately 50 cells.

9904 P

Site of Origin of Syphilitic Reagin in Spinal Fluid of Patients with Neurosyphilis.*

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Two theories have been offered to account for the presence of syphilitic reagin in the spinal fluid of patients with neurosyphilis: (1) that the reagin is derived from the blood by filtration due to impairment of the normal physiologic barrier,[1] (2) that the reagin is largely formed locally.[2]

It is generally accepted by immunologists that the syphilitic reagin is a true antibody, this belief being supported by the parallel behavior of syphilitic reagin and other antibodies both in the test-tube and in the body.[3] As an example of the latter, and in relation to the present problem, may be cited the conditions under which syphilitic reagin and other antibodies occur in umbilical cord blood. Thus, it has been shown that the isoagglutinins are not developed in newborn infants, and any present in such serum were derived from the mother by filtration through the placenta. In the course of a few weeks or months these "borrowed" isoagglutinins disappear and then the child forms its own.[4] The behavior of the syphilitic reagin is exactly parallel: a positive Wassermann or flocculative reaction on umbilical cord blood or the blood of newborn infants being diagnostic of syphilis in the mother and not necessarily in the child.[5]

Accordingly, one method of attacking the problem whether or not syphilitic reagin present in the spinal fluid was derived from the blood by passive transfer is the comparison of the behavior of the reagin with that of the isoagglutinins.

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* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Dujardin, B., *Ann. d. Mal. Vén.*, 1920, **129**, 129.

[2] For review of literature, see: Eagle, H., *Laboratory Diagnosis of Syphilis*, C. V. Mosby Co., St. Louis, 1937.

[3] Sachs and Weil, *Deutsche med. Wchnschr.*, 1925, **51**, 589; Sachs and Klopstock, *Deutsche med. Wchnschr.*, 1927, **53**, 394. For additional bibliography, see Eagle, *loc. cit.*

[4] Wiener, A. S., *Blood Groups and Blood Transfusion*, p. 19, C. C. Thomas, Springfield, 1935.

[5] Fildes, *J. Obst. and Gyn. Brit. Emp.*, 1915, **27**, 124; Faber, H. K., and Black, W. C., *Am. J. Dis. Child.*, 1936, **51**, 1257.

TABLE L

Titers of Isoagglutinins and Syphilitic Reagins in Blood Serum and Spinal Fluid of Patients with General Paresis

Blood of	Tested with	Dilution of Blood Serum									Dilution of Spinal Fluid				
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:1	1:2	1:4	1:8	1:16
R W Group O	A₁	++++	++++	++++	++++	+++	++	+	–	–	–	–	–	–	–
	B	++++	++++	++++	+++	+++	++	–	–	–	–	–	–	–	–
	e.k.*	++++	++++	++++	++++	+++	+	–	–	–	++++	++++	++	–	–
R E Group O	A₁	++++	++++	++++	++++	++++	+++	++	Tr.	–	–	–	–	–	–
	B	++++	++++	++++	+++	+++	+	–	–	–	–	–	–	–	–
	e.k.*	++++	++++	++++	++++	++++	++++	++++	+++	–	++++	++++	++++	+++	–
J B Group O	A₁	++++	++++	+++	++	+	–	–	–	–	–	–	–	–	–
	B	++++	+++	+	–	–	–	–	–	–	–	–	–	–	–
	e.k.*	++++	++	–	–	–	–	–	–	–	++++	+++	–	–	–
O.R. Group B	A₁	++++	++++	++++	++++	+++	++	–	–	–	–	–	–	–	–
	e.k.*	++++	+++	–	–	–	–	–	–	–	++++	+++	–	–	–

*Kline exclusion emulsion.

Parallel titrations were made with the sera and spinal fluids of a series of patients with neurosyphilis in order to determine their content of reagin and isoagglutinins. In order that the findings on serum and spinal fluid might be comparable, identical technics were used in testing both sorts of fluids. In the tests for isoagglutinins, known cell-suspensions, A_1 and B, were used; the test antigen for determining the reagin-content was the so-called Kline exclusion-emulsion for heated serum.[6]

Table I summarizes a few representative experiments. If the syphilitic reagin in spinal fluid were derived by filtration from the serum, there should be a simultaneous filtration of the isoagglutinins. (Naturally, this statement is based upon the not unreasonable assumptions that the molecules of syphilitic reagin and of isohemagglutinin do not differ significantly in size, and that they are sufficiently alike in electric charge so that the blood-brain-barrier would not be likely to pass selectively only one of the antibodies.) Therefore, the ratio between the titers of the reagin in serum and spinal fluid should be approximately equal to the ratio between the titers of the isoagglutinins. Actually it was found that whereas the titer of the reagin in the spinal fluid not infrequently equalled the titer in the serum, in no instance could an appreciable amount of isoagglutinin be demonstrated in the spinal fluid even when the titer of the isoagglutinin in the serum was higher than that of syphilitic reagin.

These findings cannot readily be explained by the theory of passive filtration, and support the assumption that the syphilitic reagin in the spinal fluid of patients with neurosyphilis is at least in large part formed locally.

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[6] For details of technic, see: Kline, B. S., *Microscopic Slide Precipitation Tests for Diagnosis and Exclusion of Syphilis*, Williams and Wilkins Co., Baltimore, 1932; Wiener, A. S., *J. Lab. and Clin. Med.*, 1937, **22**, 1062.

9905 P

Quantitative Studies on the Effect of X-Rays on Neoplastic Cells.*

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The contradictory reports on the effect of X-rays on cells of experimental tumors induced us to study the effect of X-rays quantitatively. Inbred mice and neoplasms that can be transmitted by the introduction of a few cells have been used in our studies.[1, 2]

The cell suspension was X-rayed in thin walled Carrel flasks. The factors of the irradiation were as follows: 130 K.V., 15 MA, 25 cm distance, 140 r/min; filter = 3 mm aluminum.

TABLE I.

		No. of mice				
		Cell suspension				
			Exposed to			
No. of cells inoculated		Not irradiated	500 r	1000 r	2000 r	4000 r
1,000,000	Injected	—	6	8	12	12
	Successfully injected	—	3 (38)	1 (92)	0	0
10,000	Injected	11	8	8	—	—
	Successfully injected	11 (13)	0	0	—	—
100	Injected	12	8	—	4	—
	Successfully injected	9 (19)	0	—	0	—
1	Injected	12	—	4	—	—
	Successfully injected	1 (82)	—	0	—	—

The figures in parentheses give the average length of life in days, after inoculation.

Table I, a summary of 3 experiments with lymphatic leukemia of Strain Ak 5, indicates that the overwhelming majority of the lymphoid cells of this strain are destroyed by 500 r of X-rays administered *in vitro* and that only an occasional cell, if any, survives exposure to 1000 r.

Experiments made with the atypical cells of a mononuclear leukemia, Strain S 2[3] indicate that after irradiation with 5,000 r

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* This investigation has been aided by grants from the Lady Tata Memorial Trust and the International Cancer Research Foundation and by a Fund for the Study of Leukemia.

[1] Furth, J., and Kahn, M. C., *Am. J. Cancer*, 1937, **31**, 276.

[2] Kahn, M. C., and Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 485.

[3] Barnes, W. A., and Furth, J., *Am. J. Cancer*, 1937, **30**, 75.

numerous cells remained viable and retained the ability to produce the disease, but cells irradiated with 15,000 r produced no leukemia. In a few experiments made the lethal dose of X-rays for different types of leukemic cells tested was intermediate between those of Strain S 2 and Strain Akf 5, that is, between approximately 1,000 and 5,000 r. There are no data in the literature on the quantitative effect of irradiation such as those presented above, and it is now generally believed that *in vitro* there is no conspicuous difference in X-ray susceptibility between malignant lymphoid and sarcoma cells.[4]

In 4 experiments made with a transmissible sarcoma of mice (Strain S 31), all of 12 mice injected with tumor tissue that had been exposed to 2,000 r of X-rays developed tumors, while all of 27 mice injected with tumor tissue exposed to from 4,000 r to 15,000 r remained healthy.

Tissue culture studies confirmed the findings of Bohn, Spear and others,[5] indicating that the destructive effect of X-rays becomes manifest only in the successive generations of cells. Unirradiated fragments of sarcoma cultured *in vitro* from 4 to 8 days readily produced tumors when injected in mice. Fragments of the same tumor irradiated *in vitro* in 2 of the above experiments with 4,000 r and 15,000 r of X-rays respectively and grown *in vitro* for a similar length of time failed to produce tumors when injected into mice. In one experiment a culture of the unirradiated sarcoma could be subcultured after 6 days but that exposed to 5,000 r units of X-rays failed to grow on subculture though it showed active growth immediately after irradiation.

A chicken sarcoma (Strain 11) transmissible by a filterable agent and exposed *in vitro* to 15,000 r units, produced tumor in 11 of 12 chickens grouped in 2 experiments. Tissue cultures were made with irradiated and unirradiated fragments of this and of another tumor induced by virus (Strain 13). The fragments of Strain 11 were exposed to 15,000 r, those of Strain 13 to 4,000 r of X-rays. The cultures from the irradiated tissues degenerated after approximately 14 days. The unirradiated tumor can be grown almost indefinitely. These experiments suggest that the virus in tissues exposed to 15,000 r is resistant to X-rays (*Cf.* 4).

Summary. The experiments described indicate conspicuous differences in susceptibility to X-rays among different neoplastic cells of mice. They support the opinion that X-rays so injure cells that multiplication can proceed only for a few cell generations, after which they degenerate and die. Viruses producing neoplasms are far more resistant to X-rays and it seems probable that exposure *in vitro* to approximately 15,000 r units of X-rays distinguishes between virus-containing and virus-free neoplasms, the latter losing the ability to transmit the disease when exposed to 15,000 r units of X-rays *in vitro*. Viruses are unaffected by the amount of irradiation used in these studies. Irradiated sarcoma cells multiply for several days and then die. Hence, failure to produce the disease with irradiated neoplastic cells is the best evidence available thus far that the neoplasms of mice studied are not transmitted by viruses.

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[4] Concerning the literature on radio-resistance of viruses and leukemic cells of mice, *cf.* Jørgensen, O. K., *Acta radiol.*, Suppl. 29, 1936.

[5] *Cf.* Spear, F. G., *Proc. Roy. Soc.*, 1930, **106**, 44.

9906 P

Calcium Studies in the Newborn.

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The purpose of this investigation was to study the relationship between the calcium of the cord blood and that of the peripheral blood during the first 10 days of life. If a low cord blood calcium regularly were associated with a low calcium during the first 10 days of life, then hypocalcemic tetany of the newborn could be predicted and prevented. In the 47 cases thus far examined there is a rather constant relationship between the cord and the postnatal blood though not of the nature mentioned above. There is a tendency for the serum calcium to assume values lower than the cord blood during the first 4 days of life and then to rise toward the prenatal level during the next 5 days.

The average calcium of the serum of cord blood was 11.60.

The average deviation of peripheral blood from cord blood of the same individual was

—1.04 for the 1st to 2nd days (11 cases)
—1.21 " " 3rd " 4th " (16 ")
—0.36 " " 5th " 6th " (8 ")
+0.55 " " 7th to 9th " (12 ")

While this work was in progress Bakwin[1] reported similar findings, based on data obtained from different sources. Our figures were obtained from the cord and postnatal blood of the same babies, whereas Bakwin compared the results of 67 examinations made during the first 10 days of life with calcium determinations on 300 cord bloods performed some years previously.

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[1] Bakwin, H., *Am. J. Dis. Child.*, 1937, **54**, 1211.

Serum inorganic phosphorus tests were done on a few cases but are not sufficiently numerous to warrant reporting.

Studies of other factors, inorganic phosphorus, total protein, blood cell concentration, feeding, etc., are being continued to determine what circumstances may influence the calcium level variations.

We are indebted to Dr. Alexander Martin, Head of the Pediatric Service of the Lincoln Hospital for allowing us to obtain the blood used for this study.

9907

Failure to Demonstrate Pressor Properties in Extracts of Blood from Hypertensives.

MYRON PRINZMETAL,* BEN FRIEDMAN† AND ENID T. OPPENHEIMER. (Introduced by B. S. Oppenheimer.)

From the Mount Sinai Hospital, New York City.

The theory that hypertension may be due to the accumulation of a humoral pressor substance or substances of renal origin has been supported by the finding of increased concentrations of pressor material in kidneys from patients with various hypertensive diseases[1] and from dogs with experimental renal hypertension.[1, 2, 3] Against this hypothesis is the failure to find increased pressor properties in the blood of subjects with hypertension.[4] Recent chemical studies[5] indicated that the pressor principle in rabbits' kidneys is related to the globulins. This work suggested the possibility of concentrating the hypothetical pressor substance in blood by similar chemical procedures. The present report is concerned with the vasomotor properties of extracts of human and of dog's blood containing protein fractions prepared in the following way.

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* Lionel Sutro Fellow.

† Richard and Ella Hunt Sutro Fellow.

[1] Prinzmetal, M., and Friedman, B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 122.

[2] Harrison, T. R., Blalock, A., and Mason, M. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 38.

[3] Harrison, T. R., Blalock, A., Mason, M. F., and Williams, J. R., Jr., *Arch. Int. Med.*, 1937, **60**, 1058.

[4] Friedman, Ben, and Prinzmetal, M., in press.

[5] Pickering, G. W., and Prinzmetal, M., *Clinical Science*, 1938, **3**, 211.

Approximately 100 cc of freshly drawn blood was defibrinated and centrifuged. The serum was drawn off and half saturated with ammonium sulfate, and the resulting precipitate dialyzed against tap water until free of sulphate. The dialysate was then dried in a Florsdorf-Mudd apparatus[6] and taken up in a small volume of saline. The final solution was tested by intravenous injection into small unanesthetized dogs with Van Leersum (carotid) loops, and the effect on the blood pressure noted.

Observations were made on the blood of 5 patients with persistent hypertension, and 3 dogs with hypertension due to renal ischemia.[7] In no instance was a well-defined pressor response noted.

The failure to find the renal pressor material in the blood stream may be due to a number of possible causes, such as inefficient extraction, insufficient amount of the pressor substance in the blood stream, or differences between the chemical properties of renal pressor substance in man and rabbits. It is also possible that ultimately the pressor substance may prove to have no relation to hypertension.

9908

Measurement of the Inhibitory Action of Anticoagulants.

ALBERT FISCHER AND TAGE ASTRUP.

From the Biological Institute of the Carlsberg Foundation, Copenhagen.

A method for measuring the inhibitory activity of heparin on the clotting of blood has been worked out by Fischer and Schmitz.[1] Instead of using as Howell[2] does an arbitrary effect as a unit for the inhibitory activity, this method gives a curve of the action of varying concentrations of the anticoagulant, and from the equation for this curve the activity of the substance is defined. From the activity (k) obtained in this way a unit for inhibitory substance has been defined by Astrup and Behrnts Jensen[3] as the quantity of active substance contained in one gram of a material which gives the value 1 for k under the given experimental conditions.

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[6] Florsdorf, E. W., and Mudd, S. J., *J. Immunology*, 1935, **29**, 389.

[7] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 346.

[1] Fischer, A., and Schmitz, A., *Z. Physiol. Chem.*, 1932, **210**, 129.

[2] Howell, H., *Bull. Johns Hopkins Hosp.*, 1928, **42**, 199.

This method has been modified by Chargaff, Bancroft and Stanley-Brown[4] and used in their recent work on blood coagulation. By this modification, curves for the inhibitory action of various concentrations cannot be determined as by the original method of Fischer and Schmitz (l.c.) and the necessity of determining such curves is here pointed out.

As the measurement of the clotting time of a blood plasma is far from being an ideal method, it is obvious that the accuracy is greatly increased with the estimation of an inhibition curve. For 5 different concentrations of an inhibitory dye, Chlorazol fast pink (Boot's Pure Drug), the activity was determined only from the differences between the clotting time of these solutions and the solutions containing one-half the original concentrations (Fischer and Schmitz[1]). The following values for k were obtained: 0.184, 0.247, 0.232, 0.140, 0.304. The average value was 0.221 in good agreement with the value 0.215 obtained from the slope of the straight line established when the logarithm of the clotting time was plotted against the concentration of the inhibitory substance.

It has been shown by Astrup[5] that the inhibitory action of various anticoagulants (not including the calcium-binding substances) differ not only quantitatively but qualitatively. When, for instance, solutions of different inhibitory substances, showing the same inhibitory effect on the same plasma system, are diluted to one-half the original concentrations, the inhibitory effects of these diluted solutions are not always equal. To show this, in Fig. 1, the logarithm of the clotting time has been plotted against the concentration of heparin in curve 1 and of Germanin in curve 2.

The anticoagulant action shows a qualitative difference which makes direct comparison of the activity of two such substances impossible. A comparison can only be made between known concentrations of the two substances.

It is then necessary, for comparative determination of the anticoagulating activities of various substances, to know the qualitative nature of the action of the different substances, and in this respect the determination of inhibitory curves is a valuable help.

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[3] Astrup, T., and Behrnts Jensen, H., to be published.

[4] Chargaff, E., Bancroft, F. W., and Stanley-Brown, M., *J. Biol. Chem.*, 1936, **115**, 149.

[5] Astrup, T., to be published.

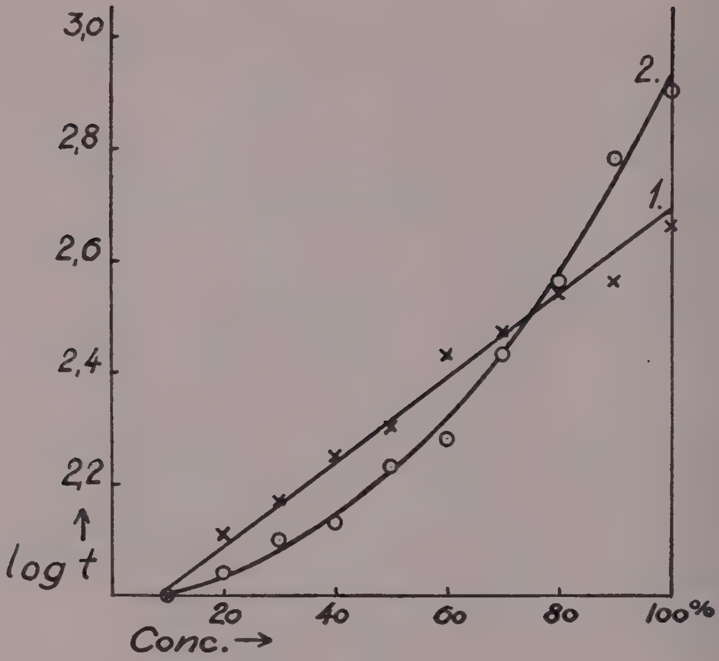

FIG. 1.
Heparin (1) and Germanin (2) in % of the original solutions.

For estimation of the activity of heparin it should be of great value in the comparison of the various methods to establish a standard for inhibitory activity. The difficulties encountered here are discussed by Astrup.[5] As the method of Fischer and Schmitz[1] yields an inhibition curve this method seems the most suitable. Furthermore, it is inexpensive and convenient to use and has proved serviceable during the past 6 years. It has been used by Dam and Schønheyder in their work on vitamin K (Schønheyder[6]).

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[6] Schønheyder, F., *Biochem. J.*, 1936, **30**, 890.

9909

Ergotamine Tartrate in Experimental Vascular Hypertension Associated with Increased Intracranial Pressure.

M. A. Lindauer, J. Q. Griffith, Jr.,* and W. A. Jeffers.
(Introduced by O. H. P. Pepper.)

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Following the intracisternal injection of colloidal kaolin, the rat will develop arterial hypertension associated with increased intracranial pressure.[1, 2] For the sake of brevity, we shall refer to such an animal as a kaolin-hypertensive rat. The elevation of blood pressure was interpreted as being the result of hyperactivity of the vasoconstrictor center in consequence of the increased intracranial pressure.

Because ergotamine is known to paralyze peripheral parts of the sympathetic nervous system, it seemed possible that this substance might influence arterial hypertension so produced. Hogler[3] has reported no lowering of the blood pressure by ergotamine tartrate when given in the dose of one mg to dogs made hypertensive by kaolin. However, Hogler's first blood pressure readings were taken one hour after the injection of ergotamine tartrate so that a transitory effect might have been overlooked.

In addition to producing prolonged hypertension associated with increased intracranial pressure, we also elicited an acute type by introducing physiologic saline into the cisterna magna at a measured pressure. This afforded a second type of test animal for investigating the effect of ergotamine.

Normal and kaolin-hypertensive albino rats were used. Light, uniform ether anesthesia was employed for all procedures. Blood pressure determinations were made by an indirect method previously described.[4] Ergotamine tartrate† was given intraperitoneally in the dose of 0.05 mg per 100 gm body weight.

Ten normal rats were injected with ergotamine tartrate, the blood pressure being followed for approximately 10 minutes before and for at least 30 minutes after the injection. No effect on blood pressure was noted.

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* Atwater Kent Fellow in Medicine.

[1] Griffith, J. Q., Jr., Jeffers, W. A., and Lindauer, M. A., *Am. J. Physiol.*, 1935, **113**, 285.

[2] Griffith, J. Q., Jr., Jeffers, W. A., and Lindauer, M. A., *Am. J. Physiol.*, 1935, **113**, 1.

[3] Hogler, *Klin. Wchnschr.*, 1934, **13**, 241.

[4] Griffith, J. Q., Jr., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 394.

† The ergotamine tartrate was supplied by the Sandoz Chemical Works, Inc.

Thirteen kaolin-hypertensive rats were similarly treated. In every instance there was a marked but transitory fall in the blood pressure (35-160 mm Hg). (Fig. 1.)

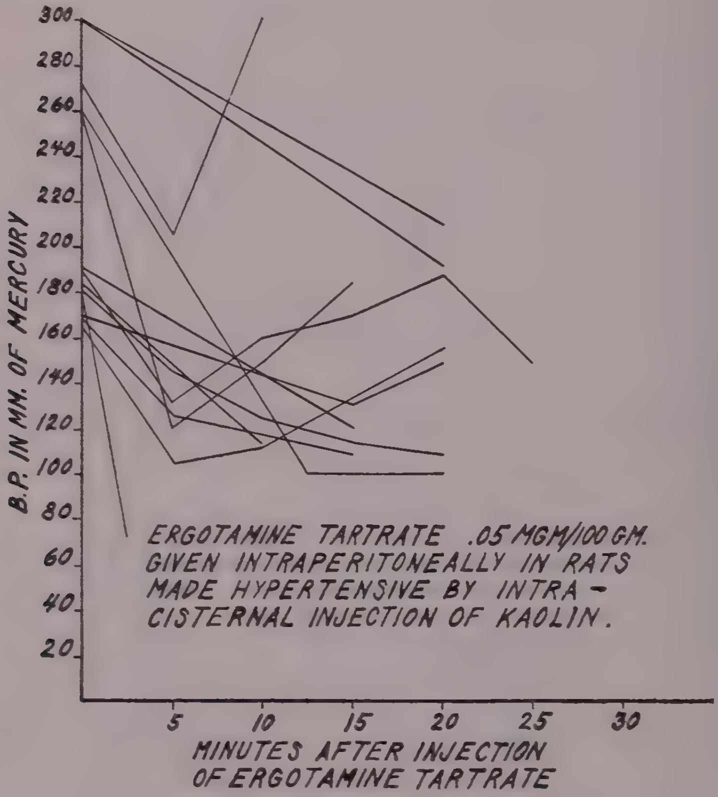

Fig. 1.

As a control, 5 kaolin hypertensive rats were given intraperitoneal injections of a solution of sodium citrate and citric acid of the same pH as the ergotamine tartrate solution (pH 4.2) and their blood pressure followed as above. One animal showed a rise in blood pressure, three showed a slight fall and one a more marked fall (an immediate drop of 23 mm Hg) but this one had been under ether for 21 minutes before the intraperitoneal injection was given.

In 3 normal rats, cerebrospinal fluid pressure was raised to 200-

270 mm of water by introducing physiologic saline into the cisterna magna. The blood pressure rose from 120-130 to approximately 200 mm of Hg. After ergotamine tartrate, all 3 showed an abrupt fall in the blood pressure, 2 dying in 1 to 2 minutes, while in the third, the blood pressure fell from 190 to 100 in 4 minutes, but in the next 12 minutes, rose steadily to the original 190, where it remained for another 10 minutes, after which the experiment was discontinued.

Electrocardiograms were taken on 4 kaolin-hypertensive rats before and after the administration of ergotamine tartrate. There was no immediate slowing of the heart rate. Three of the rats showed slowing (85-200 beats, 15-33%, per minute) 20-30 minutes after ergotamine was given.

Ergotamine tartrate lowers transiently the high blood pressure associated with increased intracranial pressure in the rat. A peripheral vasomotor depression or paralysis, which ergotamine tartrate has been shown to induce under various circumstances, and which may become obvious only in the presence of increased sympathetic constrictor activity, would appear to be a likely explanation.

The electrocardiographic findings were interpreted as evidence that the immediate fall in blood pressure was not due primarily to a cardiac depressant effect of ergotamine.

9910 P

Effect of Adrenal Vein Ligation and Pancreatectomy on Metabolism of Renal Tissue.

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Previous reports[1, 2] reveal that ligation of the lumbo-adrenal veins results in an amelioration of experimental diabetes as evidenced by diminished glycosuria and ketonuria and a basal R.Q. elevated above the diabetic level. The present experiments were conducted in order to determine the ability of renal cortex to utilize carbohydrate. Renal tissue from diabetic animals is incapable of utilizing added glucose

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[1] Fazekas, J., Himwich, H. E., and Martin, S. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 361.

[2] Fazekas, J., Himwich, H. E., and Martin, S. J., *Science*, 1938, **87**, 144.

and the respiratory quotient remains close to 0.7. The results obtained on the ligated depancreatized animals are presented in Table I. It will be noted that the basal R.Q.'s on the tissues of dogs 3 and 4 were definitely above 0.7. In all the experiments, glucose produces a questionable rise in oxygen consumption and R.Q., while lactate causes a significant increase in both oxygen uptake and respiratory quotient. The level of the blood sugar at the time the kidney was excised did not influence the character of the result. Previous work on hypophysectomized depancreatized animals failed to reveal an elevated basal R.Q.[3] and the addition of glucose failed to stimulate metabolism but lactic acid was oxidized. Further experiments are now in progress to determine whether a larger series of kidneys excised from hypophysectomized depancreatized animals would yield results similar to those obtained from the depancreatized preparation with lumbo-adrenal veins ligated.

TABLE I.

Dog	Survival, days	Blood* glucose, mg %	No addition		Glucose		Lactic Acid	
			$-QO_2$†	R.Q.	$-QO_2$	R.Q.	$-QO_2$	R.Q.
					18.1	.74		
1	14	+385	17.0	.70	18.3	.72	19.3	.79
2	10	314	17.5	.74	19.0	.79	23.2	.85
					19.1	.85		
3	25	68	17.6	.82	19.6	.81	21.3	.86
					15.6	.88		
4	25	296	14.3	.85	15.5	.87	18.5	.93

*Blood glucose at time of sacrifice.
†$-QO_2$ = mm^3 oxygen consumed per mg dog weight of tissue per hour.

9911

Adrenin Content of Adrenals of Cats Subjected to Anoxia.

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Effects of anoxemia upon the rate of secretion of epinephrine from the adrenals are not entirely clear, possibly because of the different experimental methods utilized by various authors. Cannon[1] has reviewed the positive evidence for increased adrenin secretion during anoxia, while Stewart[2] has presented certain conflicting results. Most work in the literature is concerned with anoxia of brief duration. Grollman[3] states that the type of anoxemia is important, as that of carbon monoxide stimulates epinephrine secretion while that of anoxia may not. The common occurrence of sympathetic effects during anoxia suggests that there is a definite effect on adrenal activity. Hartman[4] has shown that anoxic mydriasis after excision of the superior cervical ganglion is prevented by adrenalectomy.

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[3] Fazekas, J., Campbell, E. H., Jr., and Himwich, H. E., *Am. J. Physiol.*, 1937, **118**, 297.

[1] Cannon, W. B., *Endocrinology and Metabolism*, New York, 1924, **2**, 174; Cannon, W. B., and Hoskins, R. G., *Am. J. Physiol.*, 1911-12, **29**, 274.

The present paper is concerned only with the amount of adrenin present in the adrenals of cats after prolonged treatment at low tensions of oxygen. This approach admittedly offers no direct evidence of altered rate of secretion of epinephrine during the anoxia, since the results may be alternatively explained as effects on the rate of formation of epinephrine. It does, however, offer data which may be of aid in interpreting results of secretion studies. Previous studies[5] of the effects of anesthesia on the adrenin content of the adrenals also suggested the importance of determining the effects of simple anoxia.

Forty cats were used, of which half furnished control observations. In all except one case (see table), the cats were paired to be of similar weight, and both animals of each pair were treated at the same time, one as a control and the other as an anoxic animal. All were kept in the laboratory under identical conditions for at least 3 days before use, and other precautions recommended by Elliott[6] were rigidly followed. The anoxia-treated cats were placed in a chamber, previously described,[7] where they remained for 3 hours at an oxygen tension of 53 mm Hg, corresponding to an altitude of approximately 28,000 ft. Excitement and mydriasis were noted chiefly at the beginning of the anoxia, and most cats were quiet or depressed within a few minutes at this oxygen tension.

Following anoxic treatment, the cats were transected just below the diaphragm by a single blow of a heavy meat cleaver. Although this technic may not entirely prevent postmortem liberation of epinephrine from the adrenals, the circulation is interrupted so quickly that little epinephrine can be lost from the adrenals and surrounding tissue. Adrenin content of the adrenals was then determined by a method previously used.[5] This adaptation of the Moodey colorimetric method has been found to yield results closely paralleling those obtained by bioassay methods.[8] Control animals were treated similarly in all respects except that they were kept under normal conditions in the laboratory.

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[2] Stewart, G. N., *Endocrinology and Metabolism*, New York, 1924, **2**, 127; Pearlman, I., and Vincent, S., *Endocrinol.*, 1919, **3**, 121.

[3] Grollman, A., *The Adrenals*, Baltimore, 1936.

[4] Hartman, F. A., *Endocrinology and Metabolism*, New York, 1924, **2**, 101.

[5] Emerson, G. A., *Anesthesia and Analgesia*, 1938, **17**, 109.

[6] Elliott, T. R., *J. Physiol.*, 1912, **44**, 374.

[7] Van Liere, E. J., *Am. J. Physiol.*, 1927, **82**, 727.

TABLE I.
Adrenin Content of Both Adrenals of Anoxemic Cats.

Controls			Anoxemic		
Body wt, kg	Total Adrenin, mg	Total Adrenin, mg/kg	Body wt, kg	Total Adrenin, mg	Total Adrenin, mg/kg
2.055	.500	.243	1.745	.120	.069
1.080	.325	.301	0.950	.308	.324
1.625	.300	.185	1.580	.160	.101
1.105	.295	.267	0.930	.275	.296
2.325	.600	.258	2.655	.290	.109
0.775	.250	.323	1.780	.280	.157
1.750	.373	.213	2.255	.106	.047
2.340	.336	.144	2.390	.158	.066
1.555	.184	.119	1.510	.162	.107
1.780	.485	.272	1.470	.375	.255
1.180	.420	.356	0.960	.115	.120
3.160	.650	.206	2.360	.340	.144
1.700	.400	.235	1.410	.155	.110
2.450	.500	.204	2.350	.480	.104
1.920	.535	.279	1.510	.323	.214
2.875	.618	.215	3.030	.451	.149
0.690	.465	.674	0.880	.225	.256
2.010	.510	.254	2.250	.445	.198
3.410	.730	.214	2.450	.548	.224
0.455*	.145	.319	1.955*	.250	.128
Averages.					
1.812	.431	.264	1.821	.278	.159

*These 2 cats were used on different days; all others were paired and used as pairs on the same days.

Total Adrenin, mg/kg: *Standard error* for controls, ± 0.025; for anoxemic cats, ± 0.018. Difference between means, 0.105 ± 0.031. *Standard deviation of the series* for controls, ± 0.110; for anoxemic cats, ± 0.078.

Results are collected in Table I. The control and experimental groups were of almost the same average weight; this point is of some importance since the adrenin content is known to vary widely with the weight of the animals, particularly when it is expressed as a function of the weight. A significant difference between the means of mg per kg of total adrenin, in both adrenals, was found.

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[8] Moodey, C. B., *M.S. Thesis in Pharmacology, Univ. of Calif. Library*, 1936; Abreu, B. E., and Emerson, G. A., *Proc. W. Va. Acad. Sci.*, in press.

The results indicate, therefore, that an appreciable decrease in the adrenin content of the adrenals occurs in cats treated for a sufficiently long time at a very low oxygen tension. An additional cat subjected to an hour exposure at the same oxygen tension showed a value of 0.27 mg of adrenin per kg of body weight, which suggests that shorter exposure may not be effective; but since this value is within twice the standard deviation of the 3-hour series, the result cannot be considered significant. The possible effects from lighter degrees of anoxemia, for shorter periods, such as occur clinically during nitrous oxide anesthesia, need further investigation. Similarly, determinations of threshold degrees of anoxia and of time of exposure are beyond the scope of the present study.

Summary. Cats treated for 3 hours at an oxygen tension of 53 mm Hg show a 40% decrease in the adrenin content of their adrenals, as determined by an adaptation of the Moodey colorimetric technic.

9912 P

A Method for the Isolation of Pregnandiol from the Urine of Pregnant Mares.

PAUL G. WEIL. (Introduced by Carl G. Hartman.)

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The isolation of pregnandiol from the urine of pregnant mares has been reported by Marker, *et al.*[1] A method for the isolation of pregnandiol from the urine of pregnant mares based on a method for the quantitative determination of pregnandiol in human pregnancy urine[2] is herewith described because of its relative simplicity and possible application in the study of the function of the corpus luteum of other animals.

The fresh urine* without the addition of preservative is incubated at 37°C for 96 hours. It is filtered with suction and the precipitate is collected and heated with 100 cc of 95% ethyl alcohol. The alcoholic mixture is filtered with suction. This filtrate is evaporated to dryness and the residue taken up in 10 cc of acetone and 10 cc of a 0.1 N solution of NaOH. When complete solution is effected with moderate heating, the volume is brought to 50 cc by the addition of 30 cc of 0.1 N NaOH. The solution is placed in the cold and pregnandiol completely precipitates when the solution is thoroughly chilled. The pregnandiol is collected by filtration with suction and the precipitate is washed with warm water. The precipitate is dissolved in a minimum amount of hot acetone and 2 volumes of 0.1 N NaOH added. This is put in the cold to effect precipitation. A third precipitation using a minimum amount of ethyl alcohol and two volumes of water usually give a pure product.

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[1] Marker, R. E., Kamm, O., Crooks, H. M., Jr., Oakwood, T. S., Lawson, E. J., and Wittle, E. L., *J. Am. Chem. Soc.*, 1937, **59**, 2297.

[2] Weil, P. G., to be published.

* In this determination a liter of urine of the sixth month of pregnancy was used.

The essential points in the procedure are: (1) The liberation of the free form of pregnandiol from its conjugation form by enzyme action during incubation; (2) the insolubility of pregnandiol in aqueous solutions such as urine; (3) the purification of pregnandiol by precipitation from alkaline acetone.

9913

Effect of Nicotinic Acid, Its Isomers and Related Compounds upon Nutrition of *Staphylococcus aureus.*

MAURICE LANDY. (With the coöperation of Lester Szabo.)
(Introduced by N. Paul Hudson.)

From the Research Division, S.M.A. Corporation, Cleveland, and the Department of Bacteriology, Ohio State University, Columbus, Ohio.

Nicotinic acid has aroused widespread interest with the discovery of its rôle as a co-enzyme in tissue metabolism,[1, 2] later by the finding of its activity as a growth factor for *Staphylococcus aureus*,[3, 4] and most recently by its dramatic effect in the cure of canine blacktongue[5] and human pellagra.[6, 7, 8] In a series of unpublished ex-

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[1] Warburg, O., and Christian, W., *Biochem. Z.*, 1936, **287**, 291.

[2] Schlenk, F., and Euler, H. v., *Arkiv. Kemi, Mineral Geol.*, 1936, 12B, No. 20.

[3] Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 731.

[4] Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 966.

[5] Elvehjem, C. A., Madden, R. J., Strong, F. M., and Wooley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767.

[6] Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, J. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 405.

[7] Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. A. M. A.*, 1937, **109**, 2054.

[8] Spies, T. D., Cooper, C., and Blankenhorn, M. A., *J. A. M. A.*, 1938, **109**, 622.

periments we have been able to confirm the work of Knight[3, 4] in demonstrating the necessity of nicotinic acid and amide for the growth of *S. aureus*. Knight,[4] by testing a number of compounds closely related to thiamine, found that they could not replace it as a growth requirement of this microörganism, indicating a high degree of specificity. The importance of similar knowledge concerning compounds related to nicotinic acid is self-evident.

Using methods given in the chemical literature, we have prepared a series of compounds closely related to nicotinic acid. This series included the 2 isomers (picolinic and isonicotinic acids), sodium and ammonium salts, and ethyl ester of nicotinic acid, as well as the mono- and di-substituted nicotinic amides. The physical and chemical properties of the prepared compounds were in agreement with those cited in the literature and in general indicated a high degree of purity.

For the purpose of testing the utilization of this series of compounds by *S. aureus,* the amino acid glucose medium of Fildes, *et al.*,[9] was employed. The compounds were tested in the presence of an excess of thiamine (0.05 γ per 10 cc of medium) using an 18-hour culture of *S. aureus.*

The tabulation of the results is in Table I. All tests were repeated 2 or more times with consistent results.

The most important finding was the inability of the isomers of nicotinic acid to support the growth of *S. aureus*. The effect of substitution on the activity of the amide was marked, inasmuch as the unsubstituted amide exhibited a high degree of activity, the mono-substituted amide had a moderate effect (somewhat less than that of nicotinic acid), and the di-substituted compound manifested no activity. The contrast in the action of nicotinic acid (pyridine 3:carboxylic acid) and quinolinic acid (pyridine 2-3:dicarboxylic acid) is also of interest.

The significance of these findings with respect to tissue metabolism and animal nutrition remains to be determined.

Summary. Neither of the 2 isomers of nicotinic acid was able to replace nicotinic acid itself in the nutrition of *S. aureus*, when tested in concentrations up to 20 γ per 10 cc of medium. Reduction in the activity of nicotinic amide was brought about by introduction of an ethyl group. Complete loss of activity was evident by introduction of 2 ethyl groups into nicotinic amide. Pyridine 2-3:dicarboxylic acid was inactive as a factor in the growth of *S. aureus*.

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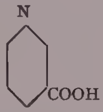
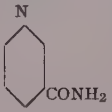
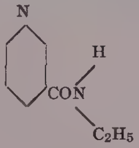
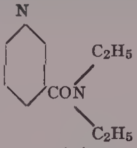
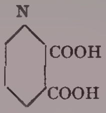
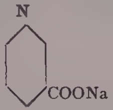
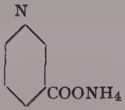
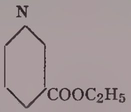
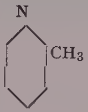
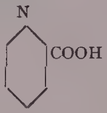
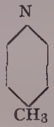
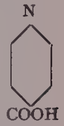
[9] **Fildes, P., Richardson, G. M., Knight, B. C. J. G., and Gladstone, G. P., *Brit. J. Exp. Path.*, 1936, 17, 481.**

TABLE I.

Effect of Nicotinic Acid and Related Compounds on Growth of *S. aureus* in synthetic medium.

Quantity of Material Tested Gamma per 10 cc of medium	 Pyridine 3-Carboxylic Acid	 Pyridine 3-Carboxylic Amide	 Pyridine 3-Carboxylic Ethyl Amide	 Pyridine 3-Carboxylic Diethyl Amide	 Pyridine 2-3-Dicarboxylic Acid	 Sodium Pyridine 3-Carboxylate	 Ammonium Pyridine 3-Carboxylate	 Ethyl Pyridine 3-Carboxylate	 2-Methyl Pyridine	 Pyridine 2-Carboxylic Acid	 4-Methyl Pyridine	 Pyridine 4-Carboxylic Acid
20	++++	++++	++++	—	—	++++	++++	++++	—	—	—	—
15	++++	++++	++++	—	—	++++	++++	++++	—	—	—	—
10	++++	++++	++++	—	—	++++	++++	++++	—	—	—	—
5	++++	++++	++++	—	—	++++	++++	++++	—	—	—	—
2.5	++++	++++	+++	—	—	++++	++++	++	—	—	—	—
1.0	+++	++++	++	—	—	+++	+++	±	—	—	—	—
.5	++	++++	+	—	—	++	++	—	—	—	—	—
.1	±	++	—	—	—	±	—	—	—	—	—	—
.05	—	+	—	—	—	—	—	—	—	—	—	—
.01	—	±	—	—	—	—	—	—	—	—	—	—

++++ = Maximum growth, other + signs proportional to amount of growth estimated by opacity. ± = Trace. — = No Growth.
Incubated for 48 hours at 37.5°C.
†0.05 gamma thiamine in each tube.

9914

Influence of Bile and Bile Salts on *Aërobacter aërogenes*.*

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Horwood and Webster[1] recently asked the question: "Is *Escherichia coli* a derivative of *Aërobacter aërogenes* in the human intestine?" The question was prompted by their study of Gram-negative lactose-fermenting bacteria from the ileostomy and fecal discharges of a patient. They found that 87.5% of the cultures from the ileostomy discharges were *Aërobacter aërogenes*, while those from the rectal discharges were all *Escherichia coli*.

The possibility that all members of the coliform group[2] of bacteria may be variants of only a few species of the group had been suggested to the writer by his experience with a group of 259 strains of coliform bacteria isolated differently from specimens of water, fish fillets, and feces. These strains had been carefully purified by approved methods. They were carried for 3 years on nutrient-agar slants, being transferred once each month to fresh media. Once each 6 months differential tests were made, the tests employed being the Voges-Proskauer, methyl-red, sodium-citrate, uric-acid, indol, and sodium-malonate. It was noted that many of the strains classified as *Escherichia coli* and as *Aërobacter aërogenes* remained constant in their reactions; but there was a pronounced tendency on the part of many strains, especially the intermediates, to vary in their reactions over the 3-year period.

The experience of Horwood and Webster, following as it did upon the author's experience, prompted the study reported here. The study was based upon the following assumption: If the intestinal environment is capable of inducing marked alterations in the biochemical behavior of *Aërobacter aërogenes*, it seemed that bile and its constituents would be likely factors in inducing these alterations. Therefore, it was the purpose of this study to determine the influence of prolonged propagation of *Aërobacter aërogenes* in media containing bile and bile salts, on the biochemical reactions of the organism.

Thirty strains were chosen for the study. They had been isolated from water, carefully purified, and carried for 3 years on nutrient-agar slants. Transfers to fresh medium had been made monthly. The identification had been made on the basis of the Voges-Proskauer, methyl-red, sodium-citrate, and uric-acid tests. In addition, all of the strains were indol-negative, and were positive to the sodium-malonate tests. These strains were chosen because of the constancy of their reactions to the tests mentioned, for the period elapsed since their isolation.

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* Published as Contribution No. 287 of the Massachusetts Agricultural Experiment Station.

[1] Horwood, M. P., and Webster, R. A., *J. Bact.*, 1937, **33**, 21.

[2] Jordan, H. E., *J. Am. Water Works Assn.*, 1937, **29**, 1999.

The medium employed was prepared as follows:

Bacto peptone	10 g
Bacto lactose	10 g
K_2HPO_4	10 g
Water	1000 ml

To this formula bile, or a bile salt, was added in the desired quantity. Bacto oxgall was employed for the bile, and sodium glycocholate and sodium taurocholate (both Pfanstiehl) were the bile salts used. The media were tubed in 10 ml quantities and sterilized at 15 lb pressure for 15 minutes.

Two series of cultures were set up, one series containing 1% and the other 5%, of the bile and the bile salts respectively. All cultures were made in duplicate, and were incubated continuously at 37°C until transferred. Cultures were transferred once each week to fresh media, and at the end of 5 months they were transferred to solid media of the same formulæ, with 1.5% agar added, to facilitate their inoculation into the differential media. This treatment was decided upon because it would have been difficult to make transfers from the fluid media without also transferring traces of the bile or the bile salts to the differential test media, and thereby possibly influencing the reactions in some of the tests.

The differential tests were those previously mentioned. In addition, lactose-fermentation, the reaction on Endo's medium, and the morphology of the organisms were rechecked. These tests were run several times during the 5 months' duration, and at the end of the experiment. All tests were carried out as recommended in the Standard Methods of Water Analysis (8th ed.), except the sodium-malonate test which is not included in Standard Methods. For this test Liefson's[3] technic was employed.

No variations were observed in any of the physiological reactions employed during the course of the experiments. The morphology of some strains was somewhat altered, the organisms appearing as long curved filaments. After transfer to ordinary nutrient-agar slants the rods regained their normal shape and size after 2 or 3 transfers at the most.

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[3] Liefson, E., *J. Bact.*, 1933, **26**, 329.

At the time of the weekly transfers it appeared that the sliminess of the culture media was much increased, particularly after the experiment had been under way for several weeks. Sliminess was especially marked in the bile and the sodium-glycocholate cultures. At the end of the experiment transfers were made into tubes of sterile milk and of lactose broth. It was found that most of the cultures gave markedly slimy (ropy) growth in both media, while only 3 of the strains were able originally to give this type of growth. However, when the strains were transferred back from the bile and the bile-salt media to nutrient-agar slants, the tendency to produce slimy growth in milk and in lactose broth was lost in all but 3 cultures originally giving that type of growth.

While the primary objective of the experiment was not to study the effect of the concentrations of bile employed on the rate of growth, it seemed desirable to determine that the growth was not interfered with to such an extent as to invalidate the results obtained. The extent of growth was checked by transfers from week-old cultures in ox-bile and bile salts into lactose broth. Serial dilutions were employed as in setting up water samples. This was repeated several times during the course of the study. The growth of all strains was vigorous enough to indicate that the bile or bile salts did not inhibit their rate of multiplication materially, if at all.

The quantity of bile employed was based on the statement of Coles and Levine[4] that a 2% concentration of bile accelerated growth of "colon bacilli", while 5% inhibited growth in an acid medium and was a stimulant in an alkaline medium. There appears to be no reference in the literature to the influence of bile on *Aërobacter aërogenes*, but since the organism usually will grow well under the same conditions as will *Escherichia coli*, the statement of Coles and Levine was used as a guide in setting up the experiments. It is known, also, that *Aërobacter aërogenes* grows well in the brilliant-green lactose-bile broth medium (Standard Methods of Water Analysis, 8th ed., 1936), which contains 2% bile.

Sufficient dipotassium phosphate was added to the media to maintain the pH definitely on the alkaline side. This was done because of the statement of Coles and Levine that 5% bile inhibited growth of the "colon bacillus" in an acid medium, and was stimulating in an alkaline medium, and in view of the fact that the recommended reaction for the brilliant green lactose bile medium is between pH 7.1 and 7.4.

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[4] Coles, H., and Levine, M., *Proc. Iowa Acad. Sci.*, 1927, **24**, 92.

It cannot be stated definitely that the characteristics of the strains employed in this study might not have been changed if the experiment had been carried on for a considerably longer time, or if greater concentrations of bile had been employed. However, in spite of these factors, it seems that the results of the experiments here reported should argue in favor of the stability of the culture reactions of pure established cultures of *Aërobacter aërogenes*, so far as the influence of bile or bile salts alone is concerned.

9915 P

Radioactive Iodine as an Indicator in the Study of Thyroid Physiology.*

S. HERTZ, A. ROBERTS AND ROBLEY D. EVANS. (Introduced by Henry Jackson.)

From the Thyroid Clinic, Massachusetts General Hospital, Boston, and the Physics Department, Massachusetts Institute of Technology, Cambridge.

The known facts of thyroid physiology indicate that iodine is selectively taken up by the thyroid gland, and that in some measure that gland's function is regulated by its iodine content. Artificial radioactivity may be induced in a variety of elements by means of neutron bombardment. It seemed that the possibility of using "tagged" (radioactive) iodine as a physiologic indicator was one which demanded investigation.

Ethyl iodide (600-1000 cc) was irradiated in a paraffin-surrounded bottle by immersing in it a neutron source consisting of 110 mg of radium mixed with beryllium in a sealed tube. The radioactive iodine thus obtained was concentrated by a method which has been described elsewhere.[1] This method gave a precipitate of radioactive silver iodide, which was dissolved in a solution of 0.5-1.0 g of sodium thiosulphate, and then diluted to 10-15 cc for intravenous injection. In a series of 48 rabbits, no toxic effects from the acute administration of such quantities were experienced. Aliquot portions of the solution of radioactive iodine used for injection were withheld for measurement of radioactivity.

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*This work was aided by a grant from the Milton Fund of Harvard University.

[1] Roberts and Irvine, *Phys. Rev.*, 1938, **53**, 609.

A Geiger-Müller counter connected to a suitable vacuum tube amplifier and register to record the individual disintegrations of the radioactive atoms was used for detection of the radioactive iodine in various tissues. This apparatus is standardized and has been fully described elsewhere.[2-4] Since no gamma rays could be observed from radioactive iodine,[1] determinations had to be made from the beta radiations. The half-period of radioactive iodine is 26 minutes, so that accurate measurements could not be extended beyond about 40 minutes after injection, with the activities available to date.

Because of the low penetrating power of beta radiation it was necessary to sacrifice the animals to obtain the various tissues for measurement. The tissues were finely minced and spread on a flat surface and placed in direct contact with the Geiger-Müller counter.

The following tissues and body fluids, in addition to the thyroid, were examined: muscle, spleen, liver, pituitary, adrenals, frontal lobe of the brain, hypothalamus, salivary glands, ovaries, blood, urine, and cerebro-spinal fluid. The results in a typical case may be given as follows:

Rabbit No. 224: Thyroid previously rendered hyperplastic by the injection of anterior pituitary extract. 10 mg of I injected. 15 minutes elapsed between injection and killing of the rabbit.

TABLE I.

Organ	Radioactivity in arbitrary units (counts per min)	
Thyroid	38.5 ± 5	
Spleen (quantity equal to thyroid)	2 ± 5	
Liver (quantity equal to thyroid)	4 ± 5	
Pituitary	0	(5 units correspond to .008 mg of I)
Urine (residue after evaporation)	48 ± 5	(In other exp. 5 units may correspond to as little as .0007 mg of I)
Muscle (quantity equal to thyroid)	0	

In none of the tissues or fluids examined were quantities of iodine found which compared with that taken up by the thyroid, with the exception of the entire bladder urine residue after complete evaporation, and the blood, into which the radioactive iodine was directly injected. Quantities less than 0.05% of the injected iodine could not be detected in these samples. It must be stressed at this point that this method gives information only with regard to the injected

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[2] Langer and Whitaker, *Phys. Rev.*, 1937, **51**, 713.

[3] Giarratana, *Rev. Sci. Instr.*, 1937, **8**, 390.

[4] Gingrich, *Rev. Sci. Instr.*, 1936, **7**, 207.

iodine, and disregards any iodine previously present within the tissues.

Other measurements of the collection of iodine by the thyroid were performed, varying the dosage of iodine, the time elapsing between injection and sacrifices of the animals, and the functional state of the thyroid under conditions of thyrotropic stimulation, pregnancy, and spontaneous goiter. The results to date of these experiments are given in the accompanying graphs. They are being extended by further experiment.

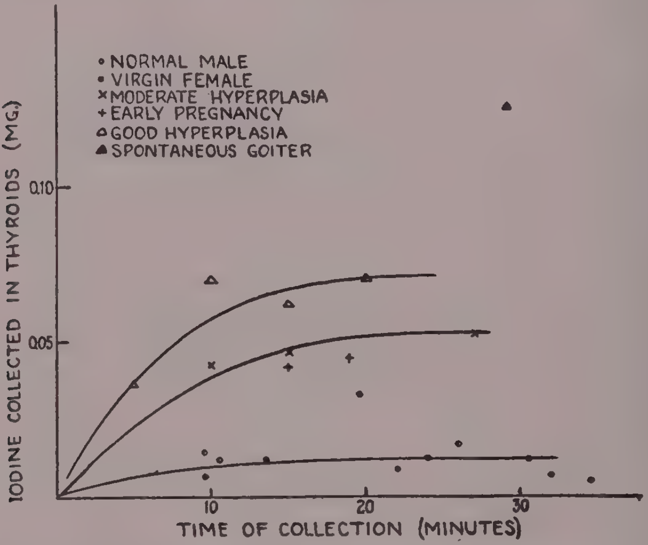

FIG. 1.

Comparison of the quantities of iodine collected by different types of rabbit thyroid glands when the time elapsing between injection and sacrifice is varied.

That the concentration of iodine in the thyroids is a biologically selective process is further attested by the uniformly negative results obtained when radioactive bromine was used, with a similar technic, in place of iodine.

The methods outlined above are established as giving useful information with regard to iodine distribution in the organism. Their accuracy can be expected to increase as sources of stronger radioactive iodine become available. The artificially radioactive material with which these experiments were performed is considered very weak, in comparison with that produced by means of the Van de Graaff electrostatic generator and the cyclotron. The data presented, however, do indicate that radioactive iodine is selectively

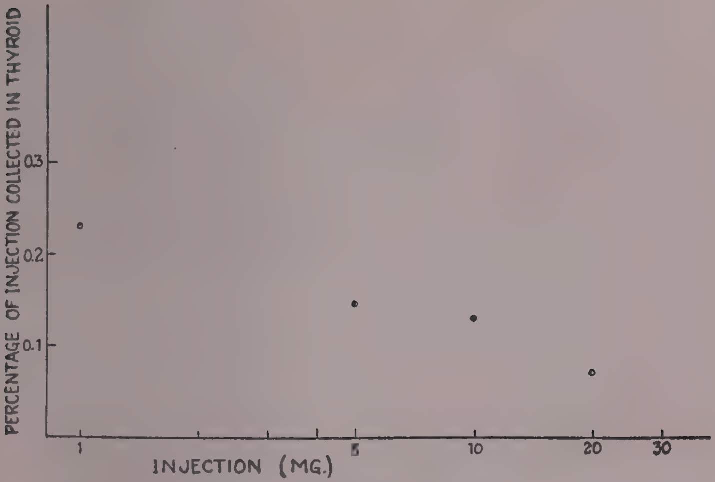

FIG. 2.

Comparison of the quantities of iodine collected by normal rabbit thyroid glands in the same time (15 minutes) when the amount injected is varied.

taken up by the thyroid under normal conditions in a definitely measurable amount. They further tend to show that under conditions of thyroid stimulation (hyperplasia), the collection in the same time is increased severalfold. It is therefore logical to suppose that when strongly active materials are available the concentrating power of the hyperplastic and neoplastic thyroid for radioactive iodine may be of clinical or therapeutic significance.

Recently the discovery of a new radioactive isotope of iodine has been announced.[5] This isotope has a half-period of 13 days. It is obvious that it will have many advantages over the 26-minute isotope for both indicator and therapeutic work. Such experiments as the above may now be extended over much longer time intervals following injection.

We are indebted to Professor J. H. Means of the Harvard Medical School for his stimulating interest in this work.

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[5] Tape and Cork, *Phys. Rev.*, 1938, **53**, 676 A.

9916 P

Gastrin.

S. A. Komarov. (Introduced by B. P. Babkin.)

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The existence of the pyloric chemical phase of gastric secretion has been sufficiently proved experimentally, but its hormonal mechanism, postulated by Edkins,[1] has been doubted. The many attempts made to establish the chemical identity of the hypothetical "gastrin" did not produce conclusive results (for literature, see Babkin[2] and Ivy[3]). Recently Sacks, Ivy, Burgess and Vandolah[4] isolated histamine from the pyloric mucosa, and concluded that "histamine is the gastric hormone, or if not, there is no gastric hormone, or the gastric hormone has never been extracted from pyloric mucosa." This view was reiterated recently by Schnedorf and Ivy.[5]

The experiments reported below demonstrate clearly that a protein-like substance, having a specific secretagogue effect on the fundic glands can be extracted in a histamine-free form from the pyloric mucosa, and in lesser quantity from the duodenum. Twenty-one experiments were carried out on cats under chloralose-urethane anesthesia, in which the gastric and pancreatic secretion, the bile flow, and occasionally also the blood pressure, following intravenous administration of various extracts prepared from different parts of the dog's stomach and intestines, were studied. The chemical procedure that was adopted for the preparation of crude extracts is as follows: The mucous membrane from different parts of the stomach of freshly killed, fasting dogs is removed with the aid of a knife and minced; intestinal mucosa is removed by scraping and ground with sand. The tissues are boiled with 10 volumes of n/10 HCl, partially neutralized with n/1 NaOH to the point where it remains only slightly acid to Congo. After cooling, the extract is centrifuged. The supernatant fluid is filtered through cotton and precipitated with 10% crystalline trichloroacetic acid. The precipitate is centrifuged and washed 3 times with 40 to 50 volumes of

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[1] Edkins, J. S., *J. Physiol.*, 1906, **34**, 133.

[2] Babkin, B. P., *Die äussere Sekretion der Verdauungsdrüsen*, 2nd ed., 1928, J. Springer, Berlin, p. 347 ff.

[3] Ivy, A. C., *Kosmos* (J. of Polish Kopernik Soc. of Naturalists), 1931, **56**, 304.

[4] Sacks, J., Ivy, A. C., Burgess, J. P., and Vandolah, J. E., *Am. J. Physiol.*, 1932, **101**, 331.

[5] Schnedorf, J. G., and Ivy, A. C., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 192.

10% solution of trichloroacetic acid in saline, twice with 50 volumes of acetone, once with benzene, and twice with ether, and dried *in vacuo*. For injection such preparations were extracted at 40°C with saline, rendered slightly acid with HCl or slightly alkaline with Na_2CO_3. These extracts were assumed to contain no histamine, choline or other extractives; some of them were tested biologically by the methods of Best and McHenry[6] and Barsoum and Gaddum,[7] and proved to be actually histamine-free. The secretagogue properties of the extracts are illustrated in Fig. 1.

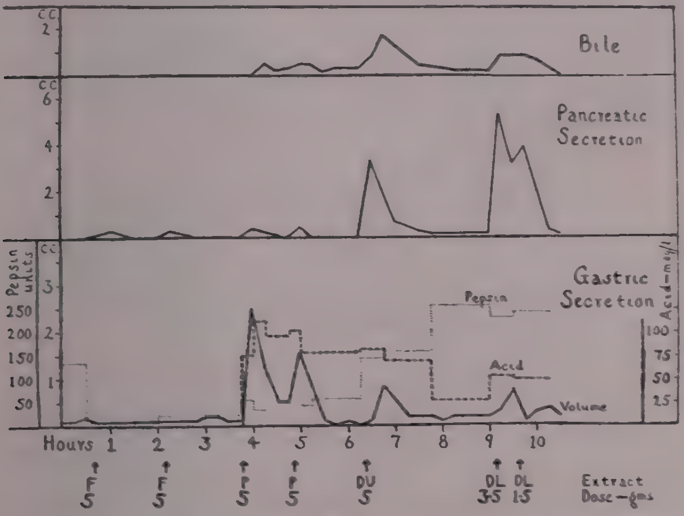

FIG. 1.

The arrows indicate the intravenous injection of extracts prepared from the mucous membrane of the fundus (F) and pyloric region (P) of the stomach, and from the upper duodenum (DU) and lower duodenum (DL). The figure below each symbol denotes the weight of mucosa equivalent to the dose of extract administered. Previous to the injection of the pyloric extract, there was only a slight secretion of alkaline mucus from the stomach.

In all cases without exception the pyloric preparations, injected in quantities equivalent to 5 gm. of mucosa, elicited a copious secretion of gastric juice, which was characterized by high acidity and low peptic power and which was not affected by atropine even in large doses. In some experiments a small pancreatic secretion and a slight acceleration in the bile flow were also observed. Fundic preparations elicited a slight secretion from the pancreas but none whatever from the gastric glands. Preparations obtained from duodenal mucosa produced a copious pancreatic secretion and a considerable increase in the bile flow; they also evoked some gastric secretion but were much less potent in this respect than the pyloric extracts. Extracts from jejunal mucosa produced a secretion from the pancreas, but none from the gastric glands.

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[6] Best, C. H., and McHenry, E. W., *J. Physiol.*, 1930, **70**, 349.

[7] Barsoum, G. S., and Gaddum, J. N., *J. Physiol.*, 1935, **85**, 1.

The active principle of the pyloric extracts was found to be soluble in 80% methyl and 80% ethyl alcohol and in dilute acetone, and to be precipitable from aqueous solutions by 10 to 30% NaCl, depending on the pH. By utilizing these properties, further purification of the pyloric principle was carried out, with resulting higher potency and complete elimination of secretin effect on the pancreas.

The above observations appear to support the "gastrin" theory of gastric secretion. They also suggest that "gastrin" may play some part in the intestinal phase of gastric secretion.

9917 P

Effect of Adrenal Cortical Hormone on Carbohydrate Stores of Fasted Hypophysectomized Rats.*

C. N. H. Long and B. Katzin.

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Russell[1] and others have shown that the 24-hour fasted hypophysectomized rat is unable to maintain its carbohydrate stores within normal limits. Furthermore, Russell[2] has found that the injection of anterior pituitary extracts, while bringing about a preservation of the muscle glycogen, does not prevent the depletion of liver glycogen and hypoglycemia.

While the effect of anterior pituitary extract in maintaining the muscle glycogen appears to be independent of the adrenals,[3] Bennett[4] has recently found that the treatment of hypophysectomized rats for some days before and during fasting with an adrenotropic extract is effective in not only maintaining the glycogen stores of muscle and liver but also in producing a slight hyperglycemia.

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* This work was materially assisted by grants from the Committee on Endocrinology of the National Research Council and the Fluid Research Fund of the Yale University School of Medicine.

[1] Russell, J. A., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 279.

[2] *Ibid., Endocrinology*, 1938, **22**, 80.

[3] Bennett, L. L., *Endocrinology*, 1938, **22**, 193.

[4] *Ibid.*, Proc. Soc. Exp. Biol. and Med., 1937, **37**, 50.

One of us[5] has suggested that the alterations observed in the pancreatic diabetes of hypophysectomized animals are largely a consequence of adrenal cortical atrophy, and strong support of this view would be furnished by a demonstration of the ability of the adrenal cortical hormone to prevent or repair the depletion of the carbohydrate stores of fasted hypophysectomized rats.

The results in Table I indicate that either cortical extract, or a crystalline compound isolated from the adrenal by Kendall[†] will not only prevent the depletion of the carbohydrate stores of fasted hypophysectomized rats but will also restore the blood sugar, liver and muscle glycogen even though they have first been lowered by fasting. In fact, in both instances, the blood sugar and liver glycogen exceed that usually found in normal rats fasted for 24 hours.

TABLE I.
Carbohydrate Levels of Hypophysectomized Rats Fasted for 24 Hours and Injected with Cortical Extract (Upjohn).

		Muscle Glycogen Mg %		Liver Glycogen Mg %		Blood Glucose Mg %	
	No.	Mean	Range	Mean	Range	Mean	Range
Untreated Normals	13	521	480-560	188	110- 310	84	72- 96
Untreated, hypophysectomized	6	320	185-413	94	57- 108	52	20- 66
Hypophysectomized, 1 cc extract every hr for 24 hr	6	440	421-498	583	263-1010	101	94-105
Hypophysectomized, .03 mg Kendall's Comp. B every hr for 24 hr	1	450	—	1520	—	107	—
Hypophysectomized, rats fasted 24 hr, then inject. 1 cc extract every hr for 12 hr	2	450	425-474	1330	1030-1570	105	104-106

In view of these results, it is difficult to avoid the conclusion that a large part, if not all, of the inability of fasted hypophysectomized rats to preserve their carbohydrate stores is due to a deficient supply of the cortical hormone. The manner in which this hormone preserves or increases the glycogen stores is being further investigated, but it is apparent that either a decreased utilization of carbohydrate or an increased production of glucose from non-carbohydrate sources would account for the effects obtained.

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[5] Long, C. N. H., *Harvey Lectures*, 1936-37, Williams and Wilkins, Baltimore.

[†] We are indebted to Dr. E. C. Kendall for this substance.

Since Russell has reported that the effect of anterior pituitary extracts in preserving muscle glycogen may be obtained in the absence of the adrenals, it is still possible that this gland secretes 2 substances affecting carbohydrate metabolism: (a) the adrenotropic principle and (b) a "glycostatic" principle which affects the tissues directly.

Summary. The injection of adequate amounts of either adrenal cortical extract or the crystalline compound B of Kendall will not only prevent the depletion of the carbohydrate stores of fasted hypophysectomized rats, but will also restore them after they have been depleted by fasting.

9918

Effect of Absolute and Partial Vitamin C Deficiency on Healing of Wounds.*

MAX TAFFEL AND SAMUEL C. HARVEY.
From the Department of Surgery, Yale University School of Medicine.

It is now well established that the intercellular substances in general, and the collagen of all fibrous tissue structures in particular require ascorbic acid for their production and maintenance. Aschoff and Koch[1] made thorough postmortem studies of World War soldiers who had died of scurvy, and demonstrated constant pathological changes in the supporting tissues of the body. Höjer[2] found a general atrophy of the connective tissue in scorbutic guinea pigs, and pointed out for the first time a general deficiency in the formation of collagen. In a series of more recent communications Wolbach[3, 4, 5] and his collaborators confirmed the observations of Höjer, and conclusively showed that ascorbic acid is intimately concerned with the synthesis and maintenance of intercellular supporting materials. Jeney and Törö,[6] when they added ascorbic acid to the nutrient medium of a culture of fibroblasts *in vitro*, found a marked increase in the number of collagen fibrils that were produced.

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* The expense of this investigation was defrayed by Davis and Geck, Inc.

[1] Aschoff, L., and Koch, W., *Skorbut*, Jena, 1919.

[2] Höjer, J. A., *Acta Pœdiat.*, 1924, **3**, 8 (supp.).

[3] Wolbach, S. B., and Howe, P. R., *Arch. Path. and Lab. Med.*, 1926, **1**, 1.

[4] Wolbach, S. B., *Am. J. Path.*, 1933, **9**, 689.

[5] Menkin, V., Wolbach, S. B., and Menkin, M. F., *Am. J. Path.*, 1934, **10**, 569.

[6] Jeney, A. V., and Törö, I., *Virchows Arch. f. path. Anat.*, 1936, **298**, 87.

Since the repair and tensile strength of soft tissue wounds are direct functions of fibroblastic proliferation and collagen formation, it is at once apparent that Vitamin C may play a prominent rôle in the phenomenon of wound healing. Saitta[7] reported a delayed healing of exposed surface wounds in guinea pigs that had been kept on a Vitamin C deficient diet for more than 15 days. On the other hand, when a Vitamin C containing extract was applied directly to the wound, the healing time was appreciably diminished, regardless of whether the animals were maintained on a normal or a scorbutic dietary regime. He determined the rate of healing by actual daily measurements of the length and breadth of the wound. Lauber[8, 9] excised square measured blocks of skin and subcutaneous tissue in experimental animals and noted the time required for complete healing and epithelialization of the wound. He found no acceleration in the healing of such wounds in guinea pigs which received subcutaneous injections of either small or large doses of ascorbic acid. The healing time of similar skin defects in 3-month-old mice was reduced by 3 days when strong concentrations of cevitamic acid were applied to the wound surface. Proto,[10] repeating Lauber's experiments in pigeons, found no significant change in the healing time of measured skin defects when Vitamin C was administered topically, orally or through both routes at once. Lanman and Ingalls[11] noted imperfect healing of operative incisions in guinea pigs maintained on a low intake of ascorbic acid. The tensile strength of wounds of the stomach and the abdominal wall of such animals, measured 10, 20, and 30 days postoperatively, was greatly inferior to that observed in normal control animals.

This series of experiments was carried out to determine the effect of absolute and partial Vitamin C deficiency on the healing of stomach wounds during the first 14 days after operation. Earlier reports from this laboratory have indicated that at the end of this period a wound in the stomach has attained almost its maximal tensile strength. The tensile strength of the wound was used as an index of healing. Young guinea pigs weighing about 250 g were divided into 3 groups: (1) normal control, (2) absolutely scorbutic, (3) partially scorbutic. All animals prior to the beginning of the experiment had been maintained almost from birth on a stock diet containing ample amounts of lettuce, kale, alfalfa, hay and celery trimmings. The scorbutigenic diet employed was a variant of the soy bean cracker meal first described by Cohen and Mendel[12] and subsequently modified by Wolbach.[3] It consisted of: soy bean meal 50, rolled oats 29, dried milk powder 10, Brewer's yeast 4, butter 5, calcium carbonate 1, sodium chloride 1. The soy bean meal was autoclaved at 15 lb for one hour. The dried milk powder was heated in a shallow pan for 3 hours at 100°C and frequently turned so as to expose all of it to the air. In addition one cc of cod liver oil was administered twice weekly by pipette. Distilled water was given *ad lib.* The diet contained adequate amounts of the necessary proximate principles, minerals, and vitamins, except for Vitamin C in which it was entirely lacking. On this diet with or without the addition of ascorbic acid the great majority of the animals either maintained their weight or increased it. Those that lost weight revealed no significant change in the bursting strength of their stomach wounds.

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[7] Saitta, S., *Scritti biol.*, 1929, **4**, 301.

[8] Lauber, H. J., *Beitr. Z. klin. Chir.*, 1933, **158**, 293.

[9] Lauber, H. J., *Beitr. Z. klin. Chir.*, 1935, **161**, 565.

[10] Proto, M., *Ann. ital. di chir.*, 1936, **15**, 31.

[11] Lanman, T. H., and Ingalls, T. H., *Ann. Surg.*, 1937, **105**, 616.

I. Control Group—31 animals. This group was maintained throughout the entire duration of the experiment on the scorbutic diet which was supplemented by daily administration by pipette of a solution containing 5 mg of ascorbic acid. (Harris and Ray[13] and later Dann and Cowgill[14] have shown that guinea pigs weighing about 250 g require about 2 mg of cevitamic acid daily as the minimum dose for full tooth protection.) The animals were kept on this regime for one week in order to equalize as much as possible the concentration of Vitamin C in the body depots. On the 8th day a longitudinal wound measuring about 2 cm in length was made under ether anesthesia through the anterior wall of the stomach at its cardiac end. The wound edges were immediately reapproximated in one layer with a running continuous Connell suture of No. 000 plain catgut which, as has been previously shown, loses its tensile strength well within the fourth day.[15] The abdominal wall was closed with 2 layers of fine No. A silk. Strict aseptic precautions were observed. On each of the 4th, 6th, 8th, 10th, 12th, and 14th postoperative days 4 to 6 animals were sacrificed, and the strength of the wound immediately determined by distending the stomach with air and noting its bursting point. The details of this method were described by Harvey and Howes.[16] Inasmuch as it has been shown that for approximately 4 days after the injury, the wounds had only the strength contributed by the holding power of the sutures, no studies were made during this first phase of healing.

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[12] Cohen, B., and Mendel, L. B., *J. Biol. Chem.*, 1918, **35**, 425.

[13] Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 2016.

[14] Dann, M., and Cowgill, G. R., *J. Nutrition*, 1935, **9**, 507.

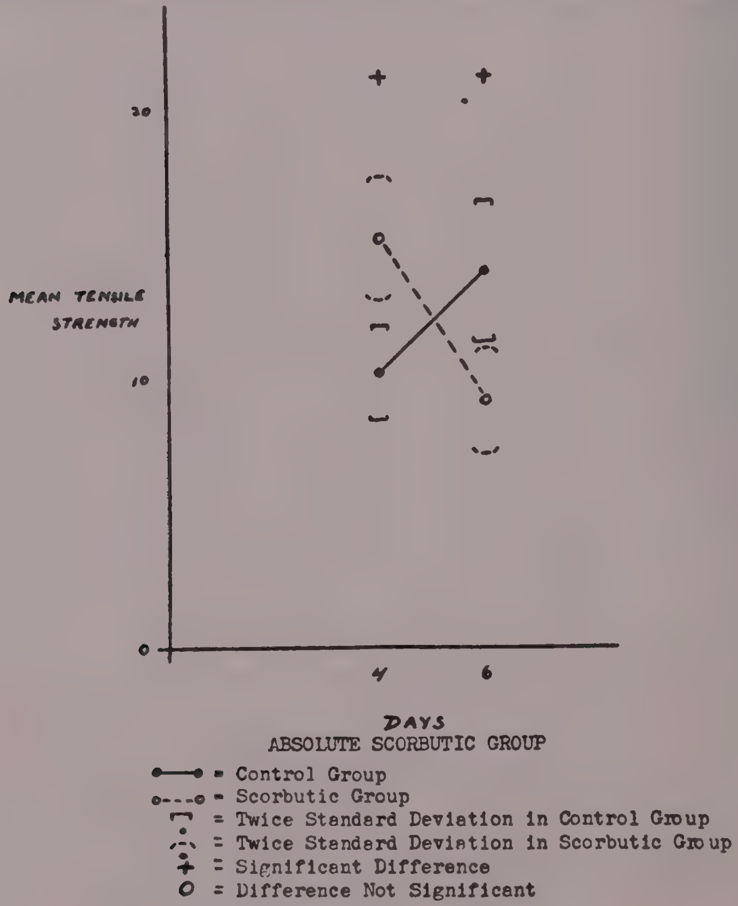
[15] Howes, E. L., *J. A. M. A.*, 1928, **90**, 530.

[16] Harvey, S. C., and Howes, E. L., *Ann. Surg.*, 1930, **91**, 641.

II. Absolutely Scorbutic Group—20 animals. This group was similarly maintained for one week on the experimental diet supplemented daily with 5 mg of ascorbic acid. On the 8th day the ascorbic acid was abruptly withdrawn. After 21 days on the scorbutigenic diet without Vitamin C the animals were operated upon, and wounds in the stomach exactly similar to those of the control group were made. At this time all animals had obvious signs of grave scurvy. The costochondral junctions and the epiphyses of the long bones, especially at the wrist and ankle, were thickened, bulbous and tender. Although only rare spontaneous fractures were noted, the long bones were so fragile that frequently even gentle handling was sufficient to crack them. Scattered small and large hemorrhages were observed in the subcutaneous tissues of the limbs and particularly on the abdominal wall where the animals rubbed themselves against the floor of the cage. At first irritable and easily excitable, the guinea pigs later became listless and apparently avoided moving about in the cage. Nearly all developed diarrhea with blood streaking of the stool. Many had a marked prolapse of the rectum. In some instances the incisor teeth became loose, but in none were hemorrhagic gums seen. The weight curve presented no constant change. Some animals lost weight, while others did not. This group at first contained 36 animals. However, after developing manifest scurvy, they died so rapidly that only 20 could be retrieved for study. Determinations of the breaking strength of the wounds could be made only on the 4th and 6th postoperative days since no member of this group survived more than 7 days after the operation.

III. Partially Scorbutic Group—34 animals. This group was also started on a one-week regime of the experimental diet plus daily doses of 5 mg of ascorbic acid. On the 8th day the ascorbic acid was withdrawn and the animals kept on the deficient diet for 14 days in order to deplete the Vitamin C in their body depots. At the end of this period they were put on a maintenance dose of 0.2 mg of ascorbic acid every other day until the termination of the experiment. After 10 days on this low Vitamin C intake, wounds were made in their stomachs. Most of the animals gained weight and survived long enough to permit determinations of the bursting strength to be carried out from the 4th through the 14th postoperative day. Postmortem examinations were made on all the animals and in each instance there were unmistakable gross evidences of scurvy. The wrist and ankle joints were beaded and the peri-articular ligaments thickened and infiltrated with old and fresh extravasations of blood. The long bones were thin and brittle. The marrow was gelatinous. There were scattered hemorrhages in the subcutaneous tissues and especially in the muscles of the thighs. In view of the obvious gross manifestations of scurvy, it was not felt necessary to get any confirmatory evidence from histological examination of the teeth and gums.

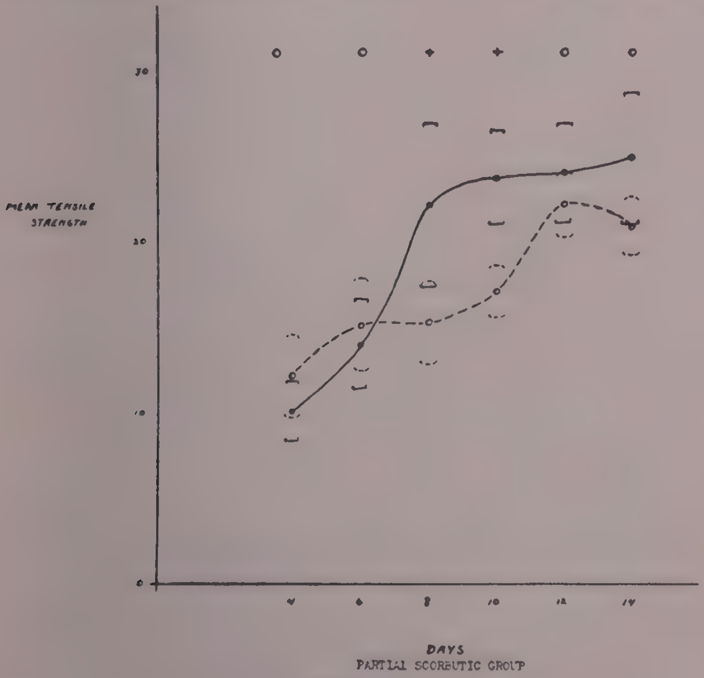
The stomach wounds of the animals in all 3 groups healed *per primam* except in 3 instances where a small walled off abscess was present at the operative site. One of these occurred in the control



GRAPH 1.

group, the other 2 in the partially scorbutic group. These 3 animals were discarded. Adhesions between the stomach and the liver or omentum were rare. In no instance were they so stubborn that they could not be easily divided to expose the healing wound. In each group the averages of the breaking strength for every postoperative interval, as well as the standard deviation were computed and plotted (Graphs 1 and 2). Fisher's[17] formula for small samples was applied to determine whether the difference in the means of 2 groups was statistically significant.

Results. In guinea pigs kept on a normal well-balanced diet, the curve of healing of stomach wounds as measured by their tensile strengths was parallel to that noted in soft tissue wounds of other experimental animals. After the fourth postoperative day there was an abrupt rise in the curve which continued until about the tenth day, when the velocity progressively decreased and slowly approached the asymptote. During this latter phase of healing some of the wounds became stronger than the surrounding uninjured



Graph 2.
Legend same as in Graph 1.

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[17] Fisher, R. A., *Statistical Methods for Research Workers*, Edinburgh, 1934.

TABLE I.

	Normal Control Group				Absolutely Scorbutic Group				
Day	No. of animals	M.T.S.*	2 S.D.†	Bursting Site	No. of animals	M.T.S.*	2 S.D.†	Bursting Site	Mathematically Significant Difference of Means
4	6	10.16	1.74	All at incision	11	15.3	2.26	All at incision	Yes
6	5	14.0	2.68	” ” ”	9	9.25	2.04	” ” ”	”

*M.T.S. = Mean of Tensile Strengths.
†2 S.D. = Twice the Standard Deviation from the Mean.

TABLE II.

	Normal Control Group				Partially Scorbutic Group				
Day	No. of animals	M.T.S.*	2 S.D.†	Bursting Site	No. of animals	M.T.S.*	2 S.D.†	Bursting Site	Mathematically Significant Difference of Means
4	6	10.16	1.74	All at incision	5	12.2	2.4	All at incision	No
6	5	14.0	2.68	” ” ”	6	15.2	2.7	” ” ”	”
8	4	22.25	4.78	” ” ”	6	15.5	2.46	” ” ”	Yes
10	4	23.75	2.74	2 elsewhere	6	17.3	1.5	” ” ”	”
12	6	24.16	2.90	5 ”	6	22.3	1.96	3 elsewhere	No
14	6	25.0	3.82	4 ”	5	20.6	1.86	1 ”	”

*M.T.S. = Mean of Tensile Strengths.
†2 S.D. = Twice the Standard Deviation from the Mean.

stomach wall, the bursting site occurring at places elsewhere than in the wound. Partial starvation, as manifested by a loss of body weight, seemed to have no effect on the progress of the healing.

In absolute scurvy, the "scorbut gravior manifestus" of Höjer, there was observed on the fourth postoperative day a mathematically significant increase in the strength of the wound over that of the control animals for the same interval. This finding was checked and found to be true in 2 separate groups of scorbutic animals. The results were pooled and expressed in Table I and Graph 1. The significance of this increase is not evident. Inasmuch as at this stage the strength of the wound is dependent almost entirely upon the extent of the fibroplasia, it is suggested that the absolute deficiency may in some way act as a stimulus for the increased proliferation of fibroblasts in the very early stages of healing. This, however, has not been proved. At the 6-day interval the strength of the wound in the scorbutic guinea pig was markedly inferior to that of the normal animal. This can be fairly attributed to the inadequate collagen formation known to occur in Vitamin C deficiency.

The healing curve in the partially scorbutic guinea pig is shown in Graph 2. On the fourth and sixth days there were no appreciable deviations from the normal. At the 8- and 10-day intervals, however, there was a significant diminution in the tensile strength, which during the 12th and 14th days again approached the normal value. This relative weakness in the wounds of the experimental group was further emphasized by a comparison of the sites of the bursting point. Out of a total of 16 control animals in the 10-, 12-, and 14-day intervals, the distended stomachs ruptured at places elsewhere than the operative incision in 11 instances. On the other hand, of 17 scorbutic animals for the same time period, in only 4 was the bursting point not at the site of the wound (Table II). The delayed healing of the wound in the partially scorbutic animal may again be directly related to the deficient or imperfect deposition and maintenance of collagen. The inferior strength of the wound at 8 and 10 days is interesting when it is remembered that clinically the great majority of wound disruptions occur during this same postoperative interval. These experiments confirm the suggestion of Lanman and Ingalls that Vitamin C deficiency in man, even when it is partial, may indeed be one of the major causative factors in those cases of wound disruption where there is no evidence of infection of the wound and where the patients for one reason or another have been on an inadequate dietary regime.

9919 P

Effect of pH on Thickness of Surface Films Made with Insulin and Other Large Molecules.

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By using suitable adaptations of the methods developed by Langmuir, Schaefer and Blodgett[1, 2] films of salmine, insulin, thymus histone, thymus nucleic acid, trypsin, and casein have been built up on chrome plated iron or on stainless steel plates. These plates were first covered with a suitable number of layers of barium stearate by the method of Blodgett.[2] They were then immersed in a 10^{-3} M solution of uranyl acetate at pH 6.0 to condition the barium stearate surface, thus rendering it hydrophilic. Each conditioned plate with an adhering layer of water was then immersed in a 0.1% solution of the substance to be adsorbed and shaken in this solution for the period of time cited. The plate was then removed, washed, and dried in air. The insulin and other surface films described below were thus formed by adsorption of molecules or molecular aggregates directly from solution and not by transfer of films previously formed on the surface of a solution. The thickness of such films has been studied as a function of a number of factors of physiological interest, such as the nature of the adsorbing surface, the time of exposure of the plates to the solutions, and the concentration, salt content, and pH of the solutions to which the plates were exposed.

The purpose of the present paper is to point out, in a preliminary way, the great influence of pH in determining the thickness of certain of these films, using typical experiments with salmine, insulin, and casein as examples. The present experiments were made at room temperature, 25°-30°C, with the salt content of the solutions kept at the minimum level.

From the preliminary data given in Table I, it may be noted that the thickness of films of salmine, insulin, or casein deposited on a conditioned barium stearate surface varied markedly with the pH of the solution from which the adsorption was made and that, with each substance, an optimum thickness was attained in the pH region near the isoelectric point of the substance in question. The thickness of films of insulin deposited on an initial salmine layer was likewise highly dependent on the pH of the insulin solution.

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[1] Langmuir, I., and Schaefer, V. J., *J. Am. Chem. Soc.*, 1937, **59**, 1406, 1762.

[2] Blodgett, K. B., *J. Phys. Chem.*, 1937, **41**, 975.

TABLE I.

Approximate thickness of salmine, insulin, and casein films deposited from solutions having various pH values. Salmine, 0.1% solution for 30 minutes. Insulin, 0.1% solution for 60 minutes. Casein, 0.1% solution for 60 minutes.

	Approximate thickness of film in Angstroms				
pH	Salmine on conditioned plate	Insulin on conditioned plate	Insulin on Salmine	Casein on conditioned plate	Casein on Salmine
4.0	15	30	10	5	25
5.0	25	70	75	400	60
6.0	35	200	500	100	50
6.5	40	400	300	75	40
7.0	50	40	150	40	25
8.0	65	20	50	—	—
9.0	75	20	—	—	—
11.*	100	—	—	—	—
12.*	75	—	—	—	—

*These values were only approximately determined.

The very thick films deposited on salmine from insulin solutions having pH values near the isoelectric point of insulin were obtained even when that fraction of the insulin which was precipitated in this pH range was removed prior to the exposure of the plates to the solution. The thickness of films of casein deposited on an initial layer of salmine varied with pH to a much smaller degree than the thickness of casein films on conditioned barium stearate.

The physiological significance of the insulin data of Table I will be briefly discussed in a forthcoming paper.[3] The extension of the present methods to the study of other cellular constituents will be more fully considered at a later date.

The authors wish to express their great indebtedness to Dr. Langmuir and his staff for the information which they have provided regarding the experimental methods of approach to the study of various types of films.

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[3] Clowes, G. H. A., to be published in Symposium of Section C, Am. Assn. for the Advancement of Science, December 28, 1937, The Science Press, 1938.

9920 P

A Study of Antigens and Antibodies by the Monolayer Film Technique of Langmuir.

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Methods have been developed by Langmuir and his coworkers[1-3] for the adsorption of dissolved substances onto the surface of suitable plates covered with multiple monomolecular layers of stearate, and for the study of various properties of these adsorbed films. Thus, through the use of simple optical techniques, the thickness of monolayers of a number of substances of biological interest (*e.g.*, egg albumin, crystalline tobacco mosaic virus protein, diphtheria toxin, chlorophyll, digitonin, sterols) has been determined.[4-6] These results check well with molecular dimensions found by other workers using different methods, such as ultra-centrifugation, filtration through collodion membranes of graded porosity, osmotic pressure measurement, etc.

With the aid of the Langmuir technic† we have made observations on the behavior of several antigen-antibody systems. The method employed consisted essentially in the preparation of Y-films of stearate made up of the desired number of layers deposited on polished chromium surfaces. A small amount of the solution under investigation was applied to the stearate surface and rubbed over it to assure adequate exposure, by passing a glass rod back and forth several hundred times in contact with the upper surface of the drop. The film was then washed, dried, and the change in thickness due to the adsorbed layer measured by comparing the interference colors of the experimental film with those of a standard Y-film stearate reference gauge, using daylight as a source of illumination.

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* James Jackson Cabot Fellow.

[1] Langmuir, I., *J. Franklin Inst.*, 1934, **218**, 143.

[2] Blodgett, K. B., *J. Am. Chem. Soc.*, 1934, **56**, 495; *Ibid.*, 1935, **57**, 1007.

[3] Langmuir, I., Schaefer, V. J., and Wrinch, D. M., *Science*, 1937, **85**, 76.

[4] Langmuir, I., and Schaefer, V. J., *J. Am. Chem. Soc.*, 1937, **59**, 1406; *Ibid.*, 1937, **59**, 2075.

[5] Blodgett, K. B., and Langmuir, I., *Physical Rev.*, 1937, **51**, 964.

[6] Langmuir, I., Schaefer, V. J., and Sobotka, H., *J. Am. Chem. Soc.*, 1937, **59**, 1751.

† One of us (M.F.S.) has had the privilege of studying these methods in the laboratory of Dr. I. Langmuir, General Electric Company, Schenectady, N. Y., through his kindness and with the encouragement of Dr. James B. Conant and Dr. Hans Zinsser.

A 2% aqueous solution of crystalline egg albumin applied to the surface of stearate wetted it readily and the area of albumin-treated stearate showed an increase in thickness of approximately 40 Å. Anti-ovalbumin rabbit serum applied to the albuminized area further increased the thickness by 100 Å. The specificity of the reactions, when immune serums were applied to antigen-treated areas in this and the other experiments, was shown by the fact that the antiserums did not adhere when applied directly to a stearate surface although they wetted it immediately. Furthermore normal rabbit or horse serums or heterologous immune serums of horse or rabbit failed to adhere to a stearate surface on which a monolayer of antigen had been deposited, although they readily wetted it.

On attempting to deposit a second layer of egg albumin on the upper surface of the anti-ovalbumin antibody already adherent to a base of albumin, the albumin solution was found to remove the antibody, leaving attached to the stearate surface only the original albumin layer. This experiment not only shows an analogy to the effect sometimes observed when an excess of antigen is added to an already existing immune serum-antigen complex, but also may indicate the relative forces existing between antigen and antibody as compared with those which bind albumin to stearate.

A 1% solution of pneumococcus type III carbohydrate (prepared either according to the methods of Heidelberger, Kendall, and Scherp[7] or of Hornus and Enders[8]) gradually wetted the stearate surface and yielded a film of 50 Å maximum thickness. Antipneumococcus type III rabbit serum applied to such an area increased the thickness by 100 Å. In contrast, the application of antipneumococcus type III horse serum to such an area increased the thickness 240 Å. Four samples of antipneumococcus type III horse serum, each of which was obtained from a different source and differed from the others in its antibody concentration, gave identical results.

Different findings were obtained, however, when similar experiments were carried out with pneumococcus types V and VIII carbohydrates and homologous antiserums. Pneumococcus type V carbohydrate in 1% concentration gave films of 50 Å thickness. Application of the homologous immune serums of both horse and rabbit to such areas gave films of 100 Å. Pneumococcus type VIII carbohydrate in a concentration of 0.4% gave films of 25 Å. Here again, application of homologous immune serums from either horse or rabbit to the treated areas further increased the thickness 100 Å.

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[7] Heidelberger, M., Kendall, F. G., and Scherp, H. W., *J. Exp. Med.*, 1936, **64**, 559.

[8] Hornus, G. J. P., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 188.

Our results with antipneumococcus type III horse and rabbit serums are in general agreement with those of Heidelberger and Pedersen[9] and Kabat and Pedersen.[10] The findings with types V and VIII antipneumococcus serums, on the other hand, strongly suggest that in the horse at least the size of the antibody protein molecule or aggregate varies according to the type of pneumococcus employed in its production. The reactions between other stearate-adsorbed antigens and their antibodies are now being investigated. A more extensive discussion of the material presented above, together with additional data, will be given in a subsequent communication.

Summary. From the above results it may be concluded that the monolayer film method provides a valuable means for the study of antigen-antibody systems as regards molecular dimensions, combining properties, and other aspects of their behavior.

9921

Effect of Cyclopropane on Intestinal Activity *in Vivo.*

C. L. Burstein. (Introduced by Co Tui.)

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In a communication reported by Peoples and Phatak[1] it was shown that in the isolated rabbit's intestine cyclopropane up to 25% in oxygen when added to the solution in which the strip of intestine is suspended causes an increase in intestinal tonus. It seemed desirable to repeat this work in the intact animal. Accordingly, experiments were conducted on normal dogs prepared with Thiry-Vella fistulas[2] of the jejunum or of the ileum.

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[9] Heidelberger, M., and Pedersen, K. O., *J. Exp. Med.*, 1937, **65**, 393.

[10] Kabat, E. A., and Pedersen, K. O., *Science*, 1938, **87**, 372.

[1] Peoples, S. A., and Phatak, N. M., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 287.

[2] Gruber, C. M., and Brundage, J. T., *J. Pharm. and Exp. Therap.*, 1935, **53**, 120.

Six dogs weighing from 10 to 16 kilos were used. Intestinal movements were recorded by inserting a balloon, connected to a water manometer as described by Jackson[3] into the lumen of the proximal end of the Thiry-Vella loop. Respiratory movements were recorded by a pneumograph applied about the chest and connected to a recording tambour. No pre-anesthetic medication was administered and all animals were anesthetized with cyclopropane and oxygen by means of a Foregger metric machine using the closed carbon dioxide-absorption technic[4] with an endotracheal airway. Guedel's[5] latest classification on depths of anesthesia was used to evaluate the degree of narcosis.

All the animals showed uniform results consisting of an increase of both intestinal contractions and intestinal tonus during the first and second planes of the third stage of cyclopropane anesthesia. During the third plane of anesthesia, tonus was still increased but contractions began to decrease. During the fourth plane of anesthesia, intestinal contractions greatly diminished while tonus remained increased. Upon lightening the anesthesia the same effects were observed in the various planes of anesthesia as those described upon deepening the degree of narcosis. The changes are recorded in Fig. 1. Intestinal contractions (waves in the kymogram) and

FIG. 1.

Effect of cyclopropane on intestinal activity in dog with Thiry-Vella fistula of the jejunum. Upper curves (A): thoracic respirations; lower curves (B): intestinal contractions.

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[3] Jackson, D. E., *Experimental Pharmacology*, C. V. Mosby, St. Louis, 1917, p. 278.

[4] Rovenstine, E. A., *Am. J. Surg.*, 1936, **34**, 456.

[5] Guedel, A. E., *Inhalation Anesthesia*, Macmillan, 1937, p. 14.

tonus (represented by the distance above the base line) are seen, in Fig. 1-b, to be increased during the second plane of anesthesia.

The effect of ether in decreasing intestinal activity[6] was also confirmed.

Conclusion. Cyclopropane causes an increase of both intestinal contractions and tone in the first 2 planes of third stage anesthesia; in the lower planes of third stage contractions are inhibited but tone is maintained. Ether, on the other hand, causes an abolition of the contractions in all planes of surgical anesthesia.

9922 P

Response of the Optic Cortex of the Cat.*

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Following a single electric stimulus applied to the optic nerve, electrical potentials of 3 types are recorded from the optic cortex (Fig. 1). The first is a series of 4 or more spikes, each of 1 m.s. duration, comparable to the spikes of peripheral axons. The second is a series of waves, each of 5 to 10 m.s. duration, and the third, a series of waves of much longer duration (Fig. 2), comparable to the alpha waves. These slower potentials do not behave like afterpotentials of the spikes. The first spike may follow a stimulus within 1.8 m.s. The following spikes occur at about 1.5 m.s. intervals, and are not simple repetitions of the first, but can be differentiated from it by differences in polarity at different depths in the cortex, by effects of anesthetics, etc. The spikes appear to represent activation of successive groups of elements rather than repetitive activation of one group. These spikes had not previously been recognized in the rabbit cortex, but with improved recording technic can now be seen to be present there also, of relatively lower amplitude. The short waves, 2 or 3 in number, correspond to those identified in the rabbit as the specific visual response. The long waves, often repetitive at 1/5 to 1/8 second intervals, correspond to the

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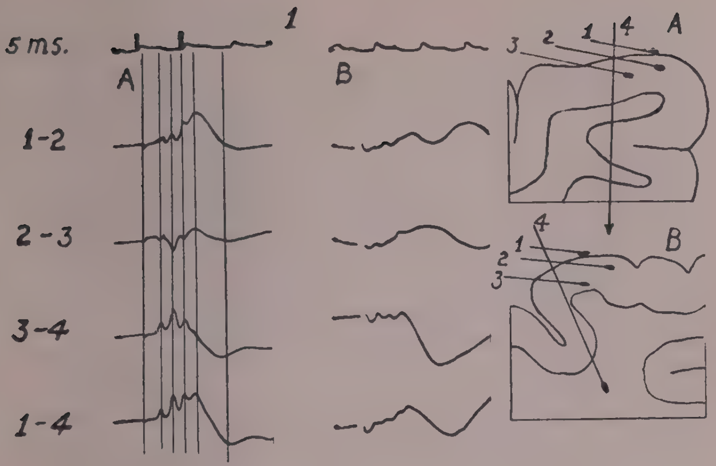
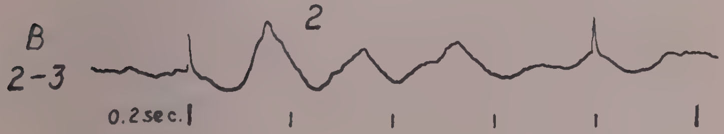
[6] Miller, G. H., *J. Pharm. and Exp. Therap.*, 1926, **27**, 41.

* Aided by a grant from the Rockefeller Foundation for research in neurophysiology.





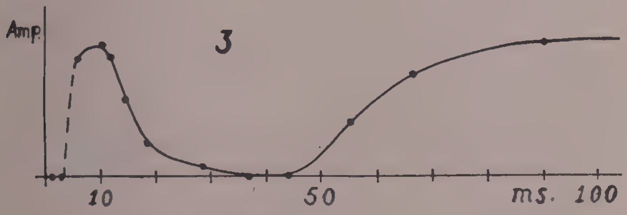


FIG. 1.

Records from upper, middle, and lower regions of the optic cortex. 1-2 etc. indicates leads from electrodes 1 and 2 of insert diagrams of cortex. A, gyrus lateralis; B, gyrus suprasylvius. Amplification 15 mm/mv except 2-3A, which is recorded at 2.5 × the amplification of the rest, corresponding to the smaller vertical distance between this pair. Deflection upwards indicates negativity at lower of electrode pair. Photographed directly on bromide paper.

FIG. 2.

Slow record of waves following those shown in 2-3B of Fig. 1. The first sharp spike represents the whole of the faster record, and is followed by a train of alpha waves at 6 per second.

FIG. 3.

Plot of amplitude of the first wave of a second response at different times after a first response, both stimuli 3 × maximal. Magnesium sulphate and ether. Without the ether and with very light $MgSO_4$, the curve does not fall to the base line at 40-50 m.s. With more ether the first hump of the curve at 10 m.s. is abolished. The second hump is repeated at the frequency of the alpha rhythm of Fig. 2.

response in the rabbit of the alpha mechanism. These 2 series of slower potentials are differentiated characteristically by anesthesia and strychnine, as in the rabbit (Bartley, O'Leary, and Bishop[1]).

We have investigated the responses of these elements to repetitive stimulation in the cat under different degrees of anesthesia. Magnesium sulphate, 0.25 g/kg or less, with ether as necessary during the preliminary operative procedures, has given the most satisfactory results. Under anesthesia sufficient to keep the animal quiet, the same phenomenon is observed as in the rabbit, that no response follows a second stimulus during the slow surface-negative phase of a first response, but a second response does follow during the positive phases of the alpha waves following this (Bartley[2]). Under lighter anesthesia, if the interval is shortened to about 25 m.s. or less, a second response also occurs, the maximum second response in this time range occurring at about 10 m.s. after the first. At this interval the second response may be of twice the amplitude of the first, both shocks being supramaximal. Ether very effectively suppresses this second response, which accounts for previous failure to observe it (Bartley[2]) even though certain summation effects below maximal suggested that both shocks were producing an effect. We thus find 2 periods of "facilitation," associated with the fast and slow responses respectively, separated by a "refractory" interval.

It may be inferred from the above that 2 optimal frequencies should be found for repetitive stimulation via the optic nerve, one at about 100 per second, which is the frequency range of rhythmic optic nerve response to retinal stimulation by light (Adrian and Matthews[3]), the other at the frequency of the alpha rhythm; and this proves to be the case. Within 3 m.s., no second response at all is obtained (Fig. 3). At a given stage of anesthesia, and with greater intervals, the first few stimuli result each in a response; the more responses occurring, the nearer the frequency falls to 100 per second, and the lighter the anesthesia. After an interval of 1/5 to 1/8 second, a second burst of responses occurs in the continuous train of stimuli, and this cycle is repeated. With very light anesthesia, each stimulus may cause a response, the amplitude varying rhythmically.

These effects of graded anesthesia suggest that in the completely unanesthetized cat, no period would be found where a stimulus would be ineffective; but that cyclic changes in irritability might manifest themselves as a modulation of the responses to repeated stimulation. Considering that photic stimulation of the retina results in repetitive responses of optic nerve fibers, a steady illumination should correspond to steadily repetitive stimulation of the optic nerve, and flickering illumination to repeated bursts of repetitive stimulation. Such bursts should be maximally effective if the individual nerve responses approached the optimum rate (100 per second for the cat), and if the bursts occurred at the frequency of the alpha rhythm.

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[1] Bartley, S. H., O'Leary, J., and Bishop, G. H., *Am. J. Physiol.*, 1937, **120**, 604.

[2] Bartley, S. H., *J. Cell. and Comp. Physiol.*, 1936, **8**, 41.

[3] Adrian, E. D., and Matthews, R., *J. Physiol.*, 1927, **64**, 279.

The accompanying paper (Bartley) contains certain evidence on the sensory effects of flicker frequency that suggests such a relationship in human subjects.

9923 P

A Central Mechanism in Brightness Discrimination.*

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Rapidly repeated flashes are seen as steady light, equivalent in sensory brightness to the same amount of light uniformly distributed in time. This is Talbot's law, and the outcome is what would be expected from a simple train of events copying the output of the photoreceptors.

But as soon as flash rate is reduced below the fusion point for steady sensation, the flashes begin to produce an average impression which is greater than that expected from photochemical considerations. As the rate is reduced more and more, the flashes produce an effect more and more nearly equivalent to steady light of the same physical intensity, and finally surpass it, reaching a maximum when the rate is reduced to the neighborhood of 8 or 10 per second. With still slower rates, the flashes approximate equivalence to steady light, with the recognized exception that single isolated flashes always appear brighter than steady stimulation of the same intensity.

The phenomenon of enhancement was first observed in 1864 by Brücke with rotating black and white sectored discs, but was not plotted for various rates of intermittency. The phenomenon shall be called the Brücke effect, though we used flashes instead of the revolving discs.

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* Aided by a grant from the Rockefeller Foundation for research in neurophysiology.

The significance of the Brücke effect is that a series of momentary stimuli is more effective for sensation than steady stimulation of the same strength. In order to account for this, the responding system must act very different from what would be expected of photoreception in the sense cells. Since the nervous system is capable of rhythmicity and periodic facilitation, we might look immediately to it. And since the maximum Brücke effect occurs at flash rates of 8 to 10 per second, such a periodicity should be sought to account for it.

The alpha rhythm in the human is of this order, and the alpha rhythm has been shown (in the cat by Bishop and O'Leary,[1] and in the rabbit by Bishop[2] and Bartley[3] to be directly connected with the periodicity of elicitation of maximum cortical response from peripheral stimulation. Stimuli given at rates not corresponding with it are able to evoke either no responses or very small ones.

There is every reason to suppose that the enhancement in the sensory effect of properly timed flashes is due to the same mechanism as underlies enhanced cortical responses from direct optic nerve stimulation after the removal of the eye, where there is no possible retinal involvement. Thus, we have here a definite case in which *brightness discrimination* is determined by a central mechanism in just as true a sense as that ascribed to photoreception.

9924 P

Spirochetes in Gastric Glands of *Macacus rhesus* and Humans without Definite History of Related Disease.

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The observation of spirochetes in the gastric glands of *Macacus rhesus* by Cowdry and Scott[1] prompted the study of the incidence of these organisms and reaction associated with or caused by their presence in the sections of the gastro-intestinal tract of the animals used in their study. Sections of human stomach taken at necropsy in the

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[1] Bishop, G. H., and O'Leary, J., *Am. J. Physiol.*, 1936, **117**, 292.

[2] Bishop, G. H., *Arch. Ophthal.*, 1935, **14**, 992.

[3] Bartley, S. H., *J. Cell. and Comp. Physiol.*, 1936, **8**, 41.

[1] Cowdry, E. V., and Scott, G. H., *Arch. Path.*, 1936, **22**, 1.

Department of Pathology of Washington University School of Medicine were made available by Dr. H. A. McCordock.

As early as 1884 Escherich[2] observed and reported spirochetes in the stools of patients with cholera. Spirochetes were described by Bizzozero[3] in the gastric glands and parietal cells of dogs in 1893.

In spite of the many reports of spirochetes in the stools of normal humans as well as in cases of so-called "spirochetal dysentery" which had been cured in many cases by treatment with arsenicals, a review of the literature fails to reveal observations or studies of these organisms in the human stomach. Mühlens[4] and Luger and Neuberger[5] have reported spirochetes in ulcerating carcinomas of the stomach but reports of spirochetes in the gastric glands or parietal cells of humans were not found.

Kasai and Kobayashi[6] experimented with lower animals which showed a definite tendency of these spirochetes of cats to become pathogenic to rabbits. Hemorrhagic erosion and ulceration of the mucosa about masses of spirochetes was described.

Edkins[7] described what he considered a granular phase of these spirochetes in cats when the cats were not fed for several hours.

In all the observations reactions to these organisms are not reported.

In *Macacus rhesus* the spirochetes were observed in the gastric glands and parietal cells (hematoxylin and eosin stained sections) in 100% of 24 control animals and 19 test animals. The marked tendency for these organisms to invade the parietal cells was noted. In most cases a few spirochetes were also seen in the neck chief cells.

In the parietal cells varying degrees of destruction ranging from mere blurring of cellular configuration to fragmentation of the nucleus and complete destruction of the distal cell margin with cytoplasmic degeneration in areas about the spirochetes were observed. Moderate degrees of cellular reaction on the part of the leukocytes have been observed about areas of massive invasion. No difference was noted between test and control animals. It is seen that even in animals that are considered normal the presence of these organisms is not always innocuous. No spirochetes were observed in the glands of the duodenum, jejunum, ileum, or colon of the same animals.

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[2] Escherich, Th., *München med. Wochenschr.*, 1884, No. 54; 1885, No. 36; 1886, No. 46; *Arztliches Intelligenz Blatt.*, 1884.

[3] Bizzozero, G., *Arch. f. Mikr. Anat.*, 1893, **42**, 82-152.

[4] Mühlens, P., *Z. f. Hyg. u. Infektionsk.*, 1907, **57**, 414.

[5] Luger, A., and Neuberger, H., *Z. f. klin. Med.*, 1921, **92**, 54.

[6] Kasai, K., and Kobayashi, R., *J. Parasitol.*, 1919, **6**, 1.

[7] Edkins, J. S., *Parasitol.*, 1920, **15**, 296.

From 3,400 necropsies 312 stomach sections, hematoxylin and eosin stained, were chosen in order as they were found. Of the 242 remaining sections, after discarding 70 on account of autolysis, 103 or 43% were found to contain spirochetes. Only 11 showed great numbers of organisms, the usual finding being a very few spirochetes after long and careful search of the entire section. These spirochetes are shorter than those in monkeys and the spirals are less sharp and few in number. The usual number of spirals was 2 or 3. Autolysis made it impossible to attach any significance to the presence of these organisms in the human stomach. Suggestive granules were observed in parietal cells in a small number of these sections.

A remarkable feature, noted by several investigators, is that such spirochetes in gastric glands are readily seen in ordinary preparations and do not require silver impregnation for their demonstration. This may be due to the special physico-chemical properties of the environment.

9925

Mental and Behavioral Changes Following Male Hormone Treatment of Adult Castration, Hypogonadism and Psychic Impotence.

NEAL E. MILLER, GILBERT HUBERT AND JAMES B. HAMILTON.
(Introduced by Edgar Allen.)

From the Department of Psychology, Yale University Institute of Human Relations, the Department of Pediatrics, Albany Hospital, and the Department of Anatomy, Yale University School of Medicine.

Previous work indicates that the administration of male hormone substance produces behavioral as well as organic changes. In baby chicks, aggressiveness, strutting and crowing have been induced.[1] In man, greater capacity for penile erection[2] and increased aggressiveness, energy and self-assurance have been noted.[3] This paper reports additional data on mental and behavioral changes during treatment with testosterone propionate* of 2 adult castrates, 2 cases of hypogonadism and 2 cases of apparently psychic impotence. Testosterone propionate was administered subcutaneously from 3 times weekly to once daily in doses of 20 mg dissolved in 1 cc of peanut oil. As controls, of which the patients were not informed, injections of oil without hormone were given for periods before and during treatment.

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[1] Hamilton, J. B., *Proc. Am. Soc. Physiol.*, 1937, 88.

[2] Hamilton, J. B., *Endocrinology*, 1937, **21**, 744.

[3] Hamilton, J. B., *Endocrinology*, 1937, **21**, 649.

* Furnished by the Ciba Company under the trade name Perandren.

Before treatment all patients were definitely lacking in any conscious goading sex drive, in the capacity for penile erection and even in the ability to enjoy sexual sensations from acts such as kissing and embracing. While the 4 patients who had once been normal could occasionally muster enough penile erection to effect vaginal entrance, these were too brief and weak to bring satisfaction to either their wives or themselves. With complete absence of either penile sensitivity or general emotional responsiveness, they reported that intercourse had become mere "arduous work."†

All patients were disturbed, anxious and broken in spirit. They ranged from moderate to severe states of mental depression. The 4 definitely organic cases displayed additional symptoms. These patients suffered from vasomotor disturbances (hot flashes), such as women may experience at the menopause, and manifested very definite emotional instability characterized by sudden uncontrollable shifts in mood, by tendencies to break down into tears, by periods of extreme irritability and, sometimes, by sullen anger. They also exhibited moderate to extreme degrees of both physical and mental fatiguability. They complained of difficulties in concentration and of general apathy.

Administration of testosterone propionate produced a marked increase in the erectile capacity and sensitivity of the penis, in the strength of the sex urge and in the capacity to respond with the proper emotions not only to intercourse but also to other acts such as kissing or embracing. During adequate treatment, the 2 castrates appeared restored to normal in these respects. While the hypogonadal cases are somewhat less clear-cut, one patient did show definite effects and the other married and eventually achieved partial orgasm. One psychogenic case (E) was restored to low normal sex drive and erectile capacity. The other patient, who had managed before treatment to escape from the environmental situation partly responsible for his difficulties, appeared to be restored to complete normality.

Normal sex function and motivation were accompanied by great changes in the entire mental attitude of all patients. Their previous despondency gave way to definite elation, which in one psychogenic case (E) tended to wear off.‡ All patients were less broken in spirit and were more spontaneous in their interests and activity. It is particularly interesting to note that additional changes occurred in the organic cases which were not observed in those without demonstrable hormonal abnormalities. The symptoms listed as peculiar to the organic cases were alleviated. They exhibited more rational aggressiveness and less irrational irritability and sullen brooding. Nervousness and emotional instability were replaced by greater stability and control. Abnormal physical and mental fatiguability disappeared. Energy and stamina returned.

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† Cases with symptoms much less severe are, of course, frequently reported.

Before treatment, none of the cases had reacted appreciably to control injections. During subsequent control periods, the one psychogenic case who had changed his environment, remained normal; but the symptoms of the other cases returned in unabated form, save that now hope was manifested instead of despair.

9926 P

Red Cell Resistance to Lysins in Pernicious Anemia.

ERIC PONDER AND C. P. RHOADS.

From the Biological Laboratory, Cold Spring Harbor, and the Hospital of the Rockefeller Institute.

Since there is evidence that there is an *in vivo* hemolytic process involved in pernicious anemia,[1] we have carried out measurements of the resistance, to saponin and other lysins, of red cells from cases of pernicious anemia in relapse and in remission.

The methods used were those described by Ponder.[2] Suspensions of red cells from normal oxalated blood and from the oxalated blood of cases of pernicious anemia are made by suspending the thrice-washed cells in sufficient 1% NaCl (or buffered Ringer at pH 7.0) to give $2.5(10^8)$ cells/cc. A series of dilutions of saponin, or of another lysin, is then prepared in 1% NaCl, and time-dilution curves for complete hemolysis are plotted at 25°C up to times as long as 300 minutes, and in some cases 600 minutes. Under these circumstances, the resistance of the cells from the blood of cases of pernicious anemia, relative to the resistance of the normal cells, is simply and properly expressed by a constant R_∞, obtained by dividing the asymptotic concentration of lysin for the cell suspension whose resistance is sought, by the asymptotic concentration for the cell suspension from the normal control ($R_\infty = 1.0$). The resistance of suspensions of normal red cells varies remarkably little.[3]

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‡ It seems probable on theoretical grounds that there should be some psychic cases which, if their impotence (*i. e.*, adjustment to need for escape or revenge, etc.) could be destroyed, might go into some other, more severe form of neurosis.

[1] Rhoads, C. P., *Cold Spring Harbor Symposia on Quantitative Biology*, 1937, **5**, 410.

[2] Ponder, E., The Mammalian Red Cell and the Properties of Hemolytic Systems, *Protoplasma Monographien*, No. 5, 1934.

In 6 cases of clinically ascertained pernicious anemia in relapse, we have found the red cell resistance to saponin to be greatly decreased, so that the time-dilution curve for the abnormal blood rises to an asymptote much higher than that for the normal blood. Frequently the 2 curves cross each other, as shown in Fig. 1, curves A and C, the times for complete lysis in high lysin concentrations being longer for the abnormal blood than for the normal, but the times in higher dilutions of lysin being very much shorter for the abnormal cells. The characteristic phenomenon, however, is the higher level of the asymptote to which the curve proceeds. To one unfamiliar with the analysis of time-dilution curves, the differences in the values of R_∞ for normal blood and for the blood from cases of pernicious anemia may not seem very impressive. As seen by the observer, however, since he is measuring the *time* required for complete lysis, the phenomenon is often very striking. Thus in a dilution of saponin of 1 in 50,000, the suspension of normal cells may hemolyse in 210 minutes while the suspension of cells from the pernicious anemia blood may hemolyse in 25 minutes; in a dilution of 1 in 100,000 the latter suspension may completely hemolyse in 5 hours, while the normal suspension may show scarcely any lysis.

In 10 clinically ascertained cases of pernicious anemia in remission, we have found the same phenomenon, although often considerably less marked (Fig. 1, curve B). Again the asymptotic concentration of lysin is less than it is for a suspension of normal red cells. It is a significant fact that the decreased resistance of the cells continues after the clinical symptoms have disappeared, and after the red cell count has risen to within normal limits. It should be pointed out that the decreased resistance can often be observed with certainty only as the asymptotes of the time-dilution curves are approached.

In 4 clinically ascertained cases of pernicious anemia in remission we were unable to detect any variation in resistance beyond the normal limits. In a few cases in which we have been able to follow the

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[3] Ponder, E., *Biochem. J.*, 1926, **20**, 507.

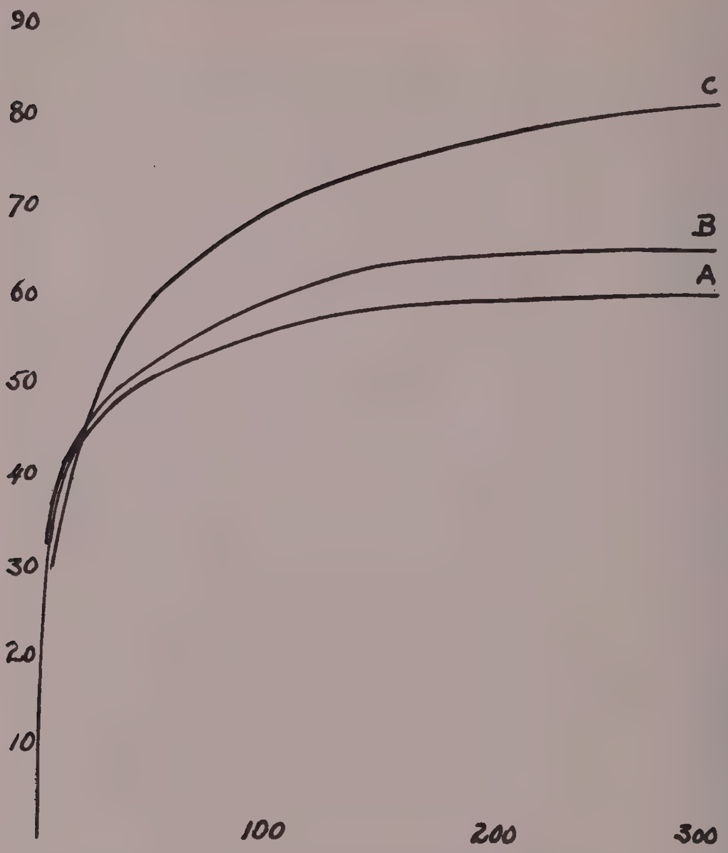

FIG. 1.

Ordinate, dilution of lysin × 10^{-3}; abscissa, time in minutes. Curve A is a typical time-dilution curve for normal blood. Curves B and C obtained in typical cases of pernicious anemia in remission and relapse respectively.

TABLE I.

Clinical condition	Red cells per mm^3	R_∞
Relapse	2,050,000	.61
”	2,160,000	.74
”	1,610,000	.62
”	1,880,000	.75
Remission	4,720,000	.84
”	4,710,000	.86
”	4,070,000	.66
”	4,720,000	.75
”	4,000,000	.97
”	3,380,000	1.00

resistance during treatment and clinical improvement, the asymptotic concentration for complete lysis unquestionably tends to fall towards the normal figure, but much more slowly than the clinical improvement takes place.

A number of typical results are shown in Table I.

These changes in resistance are observed not only with saponin (British Drug Houses), but when sodium taurocholate at pH 7.0 or sodium glycholate at pH 6.0 are used as lysins. The results clearly indicate that the red cells from the blood of cases of pernicious anemia are more vulnerable to the action of such lysins *in vitro* and probably *in vivo* also, and we are calling attention to them so that those who have access to clinical material may extend the observations.

9927 P

Cod Liver Oil Therapy in Experimental Tuberculosis.*

Horace R. Getz. (Introduced by Paul F. Clark.)

From the Department of Medical Bacteriology, University of Wisconsin.

Cod liver oil has been used from time immemorial by fisherfolk for the treatment of external wounds, and it has long been recommended for the treatment of tuberculosis. Löhr,[1] studying the wound healing properties of cod liver oil in various types of wounds, reported that this oil would aid in the healing of lupus. Our interest in the influence of cod liver oil on tuberculous lesions was especially aroused by the report of its successful use in the treatment of 3 cases of lupus by Banyai.[2]

Our purpose was (1) to confirm, if possible, the clinical observations on man by studying the effect of cod liver oil on tuberculous skin ulcers in guinea pigs, and if the results should prove favorable, (2) to isolate the chemical fraction in the oil responsible for the healing action, (3) to study its effect on other types of experimental tuberculosis, and (4) if these experiments should be satisfactory, then to determine the effect of the "pure substance" in human tuberculosis.

Tuberculous skin ulcers were produced by the intracutaneous injection of 0.2 mg of virulent tubercle bacilli (H37) and treated topically twice daily with a few drops of cod liver oil. Our first experiment, using 24 animals, verified the clinical observations in that the lesions in the treated animals healed more rapidly than those in the controls. Each animal received 3 cc of the bacterial suspension standardized by the method of Breed and Breed as given by Baldwin.[3] At 2 weeks induration was noted; ulcers approximately 1 cm in diameter were present at 3-4 weeks. Treatment was started on the 28th day of infection and lasted until the lesions were closed or until the experiment was terminated 30-40 days later. If pus was present, it was wiped out with a sterile swab both in the control animals and in the treated animals before application of the oil. This experiment has been repeated 6 times using a total of 86 cod liver oil treated animals; the ulcers have always healed faster than those of the 89 controls. (See Fig. 1.) Various other oils; namely, paraffine, lanum, olive, and cottonseed (Wesson) have been tested and all proved ineffective. Cod liver oil has been administered orally, intramuscularly, and subcutaneously, and all routes except the oral have been found effective.

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* This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

[1] Löhr, Wm., *Wundheilung*, 1937.

[2] Banyai, Andrew L., personal communication, 1935.

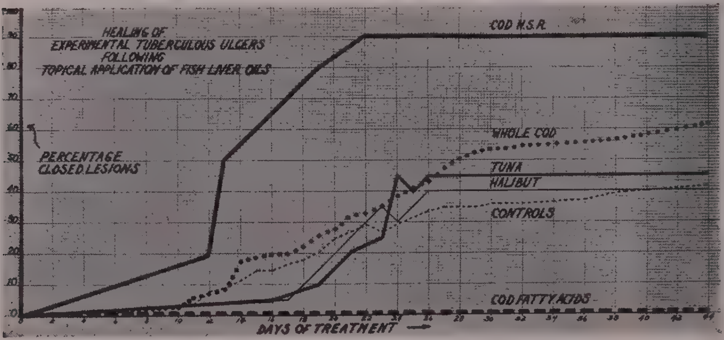

Fig. 1.

Chemical fractionation of the oil was then begun by saponification. The fatty acid fraction was found to be non-healing, in fact, quite irritating. Rancid oils produce the same reaction and so does lanum when treatment of the tuberculous ulcers is prolonged. The active agent is concentrated in the non-saponifiable residue (NSR) *since it causes healing faster than the whole oil,* often as early as the 12th day of treatment (See Fig. 1) and with remarkably little scar tissue. Early in the work it was learned that cod liver oil produces a systemic effect rather than merely a local response, as the treated animals show less generalized tuberculosis than do the controls. The NSR administered topically or intramuscularly exerts a similar but more marked effect.

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[3] Baldwin, E. R., Petroff, S. A., and Gardner, L. S., *Tuberculosis,* 1927, p. 60.

Next, the NSR was fractionated by rapidly precipitating the heavy sterols from a methyl alcohol solution with carbon dioxide snow. The precipitate of the heavy sterols was inert; and the filtrate designated the "vitamin fraction" proved to be active. This so-called "vitamin fraction" has two known constituents, vitamins A and D, and numerous unknown substances.

The investigation was continued, therefore, by testing 3 different oils† varying greatly in vitamin A and D content; halibut liver oil with 50,000 A units and 200 D units; tuna liver oil with 70,000 A units and 88,000 D units; and a control cod liver oil with 1,800 A units and 175 D units per gram. Assuming the oils to be comparable in other respects, if vitamin A were the factor, halibut and tuna liver oil should be more effective than the cod; if vitamin D were the desired agent, then tuna liver oil should induce more rapid healing. *Cod liver oil proved to be the superior healing agent* (Fig. 1), and further, the other 2 oils showed no favorable systemic effects.

Especially interesting is a recent publication by Dykes[4] reporting that cod liver oil is definitely superior to halibut liver oil in the treatment of wounds of different types.

The results here reported, using 1400 guinea pigs in 8 series of experiments, indicate that in the "vitamin fraction" of cod liver oil resides an agent which exerts a favorable healing action in tuberculous ulcers. Chemical and biological study of this fraction is being continued.

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† All of these oils were supplied through the courtesy of Dr. Warren M. Cox, Jr., of Mead Johnson and Company.

[4] Dykes, George, *Vet. Rec.*, 1937, **49**, 649.

9928

Effects on Blood Pressure of Injections of Urine Extracts of Normal and Hypertensive Individuals.

J. H. LEATHEM. (Introduced by W. W. Swingle.)

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Recent investigations indicate that the ischemic kidney contains more pressor substances than the normal kidney.[1, 2] Therefore, it appeared of interest to investigate further the possibility of an increased excretion of pressor substances in the urine of hypertensive individuals. Abelous and Bardier[3] demonstrated the presence of pressor substances in normal urine. This was substantiated by Bain[4] who, in addition, found that the urine of hypertensive individuals gave only slight, if any, pressor effect and concluded that these individuals retained the pressor substances. More recently Bohn and Hahn[5] by a modification of Bain's method reported the presence of pressor substances in the urine of pale hypertensive cases but that these materials were virtually absent in the urine from red hypertensive and normal individuals.

For these experiments, 24-hour urine samples were collected daily from 2 individuals (one male and one female) with essential hypertension having normal renal function and exhibiting a blood pressure of 200 mm Hg. Normal urine was obtained daily from students in the laboratory. The urine was extracted the same day according to the method of Bohn and Hahn by which the final residue was dissolved in Ringer-Locke's solution.

In an attempt to establish comparable test conditions, all the blood pressure tests were made on one normal unanesthetized dog which was carefully trained for blood pressure work.* Damshek and Loman's[6] intra-arterial blood pressure method was employed and

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[1] Harrison, T. R., Blalock, A., and Mason, M. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 38.

[2] Prinzmetal, M., and Freidman, B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 122.

[3] Abelous, J.-E., and Bardier, E., *C. R. Soc. Biol.*, 1908, **64**, 596, 848.

[4] Bain, W., *Lancet*, 1910, Apr. 30, 1190; *ibid.*, 1911, May 27, 1409; *Quart. J. Exp. Physiol.*, 1914, **8**, 229.

[5] Bohn, H., and Hahn, F., *Z. f. Klin. Med.*, 1933, **123**, 558.

* Grateful acknowledgment is due to Dr. W. M. Parkins for the blood pressure determinations.

[6] Damshek, W., and Loman, J., *Am. J. Physiol.*, 1932, **101**, 140.

the mean pressures determined in the femoral artery.[7] Injections were made into the jugular vein, always using the same quantity (10 cc) regardless of the urine equivalent. The injection period was 30 seconds in every case.

The initial tests were made with 10 liter concentrates, but later experiments showed that 1 to 2 liter quantities were quite satisfactory to compare the normal and hypertensive urines with this test animal. Four typical comparative tests exhibiting the similarity of the pressor activity of the urine extracts are shown in Table I. Generally, the pressure rose immediately on injection and reached its peak in 1 to 3 minutes, but in a few cases there was a short latent period. This rise was followed by a gradual decline and return to normal pressure. The effect varied between 5 and 15 minutes as determined from the time of injection. The cases surpassing 10 minutes were usually the ones that exhibited the initial latent period. An initial depression of pressure was observed in only one case. A series of tests were made using 1 liter urine equivalents as this provided the most sensitive range and blood pressure effects varying from 0 to 40 mm Hg were obtained. Although 2 extracts of normal urine gave no pressor activity the average of 24 mm Hg compared favorably with a 32 mm Hg average for hypertensive urine.

One extract was tested on an adrenalectomized dog being maintained on cortical hormone and a blood pressure rise of 40 mm Hg resulted with a 2 liter equivalent of hypertensive urine.

Bain obtained only slight pressor responses from hypertensive urine. An extract of his ether soluble fraction was prepared from 1500 cc of hypertensive urine which, on injection, produced a 40 mm Hg rise in pressure. This compared with the results obtained with the normal urine. Further treatment of the alcohol soluble fraction of Bohn and Hahn's method was attempted and it was found that neither filtration through permutit nor ether extraction would remove the pressor substances. The pressor activity was not destroyed after 7 days at room temperature.

Abelous and Bardier[8] were not able to find pressor substances in normal dog urine and therefore Bohn and Hahn's method was used in extracting dog urine. Twenty-four-hour samples were collected from 2 dogs with normal blood pressures and the extracts tested on 3 trained unanesthetized dogs. The concentration of urine was found to vary daily and thus the pressor activity of identical urine equivalents showed little agreement (Table I). However, the

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[7] Parkins, W. M., *Am. J. Physiol.*, 1934, **107**, 518.

[8] Abelous, J.-E., and Bardier, E., *C. R. Soc. Biol.*, 1908, **65**, 63.

TABLE I.
Effects on Blood Pressure of Unanesthetized Dog of Injections of Urine Extracts.

Normal pressure, mm Hg.	Max. pressure, mm Hg.	Total rise, mm Hg.	Period effective, min	Urine equivalent injected*	Urine used
Effects of Urine Extracts of Normal and Hypertensive Individuals.					
94	144	50	10	4 l	Hypertensive
98	148	50	12	4 ”	Normal
96	124	28	15	800 cc	Hypertensive
94	114	20	10	800 ”	Normal
98	130	32	8	1 l	Hypertensive
98	132	34	7	1 ”	Normal
98	136	38	5	1 ”	Hypertensive
98	128	30	6	1 ”	Normal
Effects of Dog Urine Extract.					
96	128	32	6	1 l	Normal
96	131	35	6	1 ”	”
92	100	8	5	1 ”	”
94	104	10	4	1 ”	”
98	108	10	4	1 ”	”
96	114	18	5	1 ”	”
98	108	10	5	1 ”	”
98	—	0	—	1 ”	”
94	132	38	6	1 ”	”

*10 cc injected in 30 seconds in each case.

activity of one extract when tested on 2 dogs would produce relatively the same effect and indicated that pressor substances are present in normal dog urine.

Summary. Although Bohn and Hahn found pressor substances in hypertension and Bain found this material only in the normal, the results of these experiments indicated that no significant differences in the amount of pressor substances, other than possible normal variations, existed between the urine of normal individuals and the urine of the 2 essential hypertensive individuals that were tested. Furthermore, the normal dog was found to excrete pressor substances in variable quantities.

9929 P

The Unidentified Base in Gelatin.

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DOUGLAS MACFADYEN.

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Van Slyke and Hiller[1] reported evidence that the phosphotungstate precipitate obtained from hydrolyzed gelatin contained amino acid material other than the hexone bases usually found in this precipitate. Other problems prevented continuance of work on the isolation of the material. It was taken up by Van Slyke and Robson[2] but after preparation of a copper salt, since found to be contaminated with proline, the work was again discontinued by Robson's return to England.

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[1] Van Slyke, D. D., and Hiller, Alma, *Proc. Nat. Acad. Sci.*, 1921, 7, 185.

The unidentified base has now been isolated and recrystallized, both as the picrate and as the hydrochloride, and has the composition of a hydroxylysine. After removal of arginine and histidine as silver salts the residual "lysine fraction" is freed of adherent monoamino acids by repeated precipitation in very dilute solution as phosphotungstate. The greater part of the lysine is removed by addition of enough picric acid to the hot aqueous solution to combine with ¾ of the amino nitrogen present. To the mother liquors enough more picric acid is then added to combine with the rest of the amino nitrogen. A picrate of the composition $C_6H_{14}N_2O_3 . C_6H_3N_3O_7$ is crystallized, from a dilution of about 1 to 10, and is purified by recrystallization. It is more soluble than the picrate of lysine, and when heated at the rate of a degree every 3 seconds shows a melting point at 225°. Both the nitrogens of the amino acid react completely with nitrous acid in 10 minutes. There is a marked difference from lysine in speed of reaction; in 3 minutes at 25° under the conditions of the manometric analysis[3] lysine evolves only 83% of its total nitrogen, while the new base in the same time evolves 95%.

The monochloride, $C_6H_{14}N_2O_3 . HCl$, was prepared by decomposing the picrate with HCl and ether, removing the excess HCl with Ag_2O, bringing to pH 6 with HCl, and precipitating from concentrated aqueous solution by addition of hot alcohol. The precipitate, at first amorphous, crystallized as rods. The C, H, N, NH_2, Cl, and amino acid carboxyl[4] contents were those calculated for hydroxylysine.

We are indebted to Dr. Adalbert Elek for micro-combustions.

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[2] Van Slyke, D. D., and Robson, W., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 23.

[3] Van Slyke, D. D., *J. Biol. Chem.*, 1929, **83**, 425.

[4] Van Slyke, D. D., and Dillon, R .T., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 362; *Compt. rend. Lab. Carlsberg*, 1938, **22**, 480.

9930 P

Effect of Calcium Upon Uterine Activity and Reactivity.

D. N. Danforth and A. C. Ivy.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Since the original reports by Blair-Bell[1, 2] very little experimental study has been devoted to the effect of calcium on uterine motility, although in the clinical literature varying opinions have been expressed. This study was undertaken to repeat in part the experiments of Blair-Bell and to ascertain the relation of available calcium to the reactivity of the uterus to pitocin.

Ten postpartum dogs were used for this study. Uterine motility was recorded by the method of Rudolph and Ivy,[3] in which a balloon is placed in each uterine horn, appropriately inflated, and connected to a water manometer. After control tracings had been made the following solutions were administered intravenously: calcium, as a 10% solution of calcium chloride; sodium hexa-metaphosphate, a substance which combines with calcium in such a manner that it is unable to act physiologically; and pitocin (Parke, Davis & Company). After the effects of these had been observed, they were combined, as indicated below, and administered. Sodium pentobarbital was used as an initial anesthesia. After the dog had been prepared, analgesia was maintained by the subcutaneous injection of morphine sulfate at hourly intervals. This did not affect uterine motility. The results of the experiments on the dog were checked *in vitro* by using uterine strips obtained from guinea pigs in diestrus. Either oxygenated "normal" or calcium-free Locke's solution was used.

Dog's uterus in situ. In all instances (20 experiments) calcium, in doses of from 10-30 mg of calcium per kilo of body weight, was found to augment the motility of the dog's uterus *in situ.* Both the rate and amplitude of contraction, as well as coördination between the 2 horns, were increased. The opposite effect was noted when sodium hexametaphosphate (20 experiments) 10-25 mg per kilo was administered; in many instances the uterus was reduced to a state of complete quiescence which was sustained, frequently, for more than 20 minutes. The injection of suitable amounts of

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[1] Blair-Bell, W., and Hick, P., *Brit. Med. J.*, 1909, **1**, 777.

[2] Blair-Bell, W., *Proc. Roy. Soc. Med.*, 1915, **8**, 71.

[3] Rudolph, L., and Ivy, A. C., *Am. J. Obst. and Gynec.*, 1930, **19**, 317.

calcium immediately restored normal rhythm and coördination.

The minimal effective dose (M.E.D.) of pitocin (which varied from 1 cc of a 1:10,000 solution per kilo to 1 cc of a 1:1,000 solution per kilo) was determined by injecting that amount which produced a definitely detectable increase either in rate, or amplitude of the uterine contractions, usually both. Larger doses, of course, produced tetany. When the M.E.D. of pitocin was given simultaneously with, or shortly after, the injection of calcium, the effect was invariably greater (20 experiments) than that produced by the pitocin alone. Conversely, when the M.E.D. of pitocin was given with, or shortly after, the injection of sodium hexametaphosphate, the effect was either markedly reduced or not observed at all. The latter reaction was the rule (15 experiments), the former the exception (5 experiments). After injecting an adequate dose of calcium the uterus responded to an M.E.D. dose of pitocin.

Guinea pig's uterus in vitro. When the guinea pig's uterus was placed in oxygenated Locke's solution the addition of calcium (1.5-3.0 mg to a 50 cc bath) caused augmentation. The addition of 0.05 to 0.1 cc of a 1:100 solution of pitocin augmented the contractions (12 experiments). When the 2 were added to the bath simultaneously an additive effect was observed. When the uterus was bathed (12 experiments) with oxygenated calcium-free Locke's solution pitocin in the doses given above either had no, or only a very slight effect. These results confirm those of Van Dyke and Hastings.[4]

This study substantiates previous findings concerning the oxytocic effect of calcium and, further, shows that in its absence the uterus is incapable of contracting in a normal manner or of manifesting its usual reactivity to pitocin. The activity and reactivity of the uterus under conditions in which the available calcium is diminished suggest the condition observed during early pregnancy and during the latter part of the menstrual cycle, and also following treatment with progesterone. There is no evidence showing that the two states of the uterus just referred to have a common basis, but the similarity is striking. This is now being investigated.

Conclusion. Spontaneous contractions of the postpartum dog uterus or the diestrous uterus of the guinea pig may be augmented by the administration of calcium or depressed or completely inhibited by diminishing the available calcium. In the latter instance the uterus is usually not only quiescent, but is also relatively refractory to pitocin.

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[4] Van Dyke, H. B., and Hastings, A. B., *Am. J. Physiol.*, 1928, **83**, 563.

9931

Induction of Ovulation in *Rana pipiens* by Pituitaries of *Triturus viridescens.**

A. Elizabeth Adams and Barbara Granger.

From the Department of Zoology, Mount Holyoke College, South Hadley, Mass.

Since the first report of ovulation induced during the non-breeding season in *Rana pipiens* by homoplastic anterior pituitary transplants,[1] this species has been used repeatedly for demonstrating and studying this reaction.[2] This form will also ovulate after treatment with pituitaries of other species of Rana, *e.g.*, *R. sphenocephala*, etc.,[3] and of fish, Lepidosteus,[4] and with beef pituitary extract (phyone).[5] There is, however, the report that "mammalian pituitary extract, whole sheep pituitary, and the Antuitrin-S from pregnant human female urine have proven to be entirely ineffectual in respect to ovulation in *Rana pipiens*" (p. 29[6]) and more recently negative results followed the use of pregnant mare's serum, Antuitrin-S, beef anterior lobe extract, Antuitrin, live implants and macerated injections of whole glands from fishes *(Perca flavescens, Cristovomer nanaycush, Stizostedion vitreum)* and from *Necturus* even when very large doses were used.[7]

Because of this apparent refractoriness of *Rana pipiens* to heteroplastic anterior pituitary treatment, tests were made in which whole pituitaries of *Triturus viridescens* were injected into the body cavities of females while others received equivalent amounts of homoplastic pars anterior. In a preliminary test made in December, 1937, in which 4 anterior pituitaries from male *Rana pipiens* and 8 whole pituitaries of *Triturus viridescens*, respectively, were implanted into 2 animals, no ovulation occurred, so larger doses were employed in the succeeding experiments.

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* Aided by a grant from the Rockefeller Foundation.

[1] Wolf, O. M., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 692.

[2] Bardeen, H. W., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 846; Rugh, R., *Biol. Bull.*, 1934, **66**, 22; 1935, **68**, 74; *J. Exp. Zool.*, 1935, **71**, 149, 163; *Science*, 1937, **85**, 588; *Physiol. Zool.*, 1937, **10**, 84.

[3] Creaser, C. W., and Gorbman, A., *Copeia*, 1936, No. 2, p. 91.

[4] Wills, I. A., Riley, G. M., and Stubbs, E. M., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 784.

[5] O'Neil, H., unpublished data.

[6] Rugh, R., *Biol. Bull.*, 1934, **66**, 22.

[7] Creaser, C. W., and Gorbman, A., *Am. J. Physiol.*, 1935, **113**, 32; *Copeia*, 1936, No. 2, p. 91; Creaser, C. W., and Shcolnek, M., *Anat. Rec.*, 1937, **70**, Suppl., No. 1, 117.

Rana pipiens injected with pars anterior from Rana pipiens. No. 1. 1/9/38, ♀, wt 73.9 g, received 2.42 mg P.A. from 3 ♀ R. p. (av wt, 35.5 g); 1/11, 2.43 mg P.A. from 2 ♀ R.p. (av wt, 62.2 g) were injected; 1/15, two bunches of eggs in water.

No. 2. 1/22/38, ♀, wt 88.4 g, received 4.51 mg, P.A. from 4 ♀ R. p. (av wt 58.3 g); 1/27, at autopsy, ovaries were nearly empty, 2 eggs were in body cavity, 3 in oviducts and uteri were full.

No. 3. 1/27/38, ♀, wt 93.6 g, received 7.62 mg P. A. from 6 ♀ R. p. (av wt, 85.1 g): 1/28, 1/29, 1/31, eggs pressed from cloacal opening; 1/31, at autopsy, ovaries contained 13 mature eggs, 3 were in body cavity, one in left oviduct, and uteri were very full.

No. 4. 1/31/38, ♀, wt 116.8 g, received 5.67 mg P.A. from 4 ♀ R. p. (av wt, 57.0 g); 2/2, uteri appeared full and eggs were pressed from cloacal opening, after which 3.59 mg P.A. from 2 ♀ R. p. (av wt, 77.8 g) were injected; 2/3, ♂ which had received 1.96 mg P.A. from 2 ♀ R. p. was placed with ♀; 2/4, large numbers of eggs in water; 2/7, at autopsy, 8 mature eggs in ovaries, 9 in body cavity, and a medium-sized bunch of eggs remained in left uterus.

Rana pipiens injected with whole pituitaries from Triturus viridescens. No. 5. 1/9/38, ♀, wt 77.6 g, received 3.29 mg whole pituitaries from 24 ♂ T. v.; 1/11, 3.16 mg from 16 ♀ T. v. injected; 1/18, at autopsy, ovaries were still full of eggs but 6 were found in body cavity, 2 in left oviduct, and 9 in uteri. Seven eggs were released from left ovary when it was shaken in water.

No. 6. 1/22/38, ♀, wt 92.2 g, received 6.92 mg whole pituitaries from 27 ♀ and 17 ♂ T. v.; 1/25, many eggs pressed from cloacal opening and more shed spontaneously later; 1/27, few eggs pressed from cloaca, after which animal was autopsied. Both uteri were full of eggs, 5 eggs were in right oviduct, and 2 in body cavity. Ovaries were almost empty.

No. 7. 1/22/38, ♀, wt 96.1 g, received 7.49 mg whole pituitaries from 25 ♀ and 19 ♂ T. v.; 1/27, animal killed. No eggs ovulated.

No. 8. 1/27/38, ♀, wt 95.0 g, received 11.67 mg whole pituitaries from 39 ♀ and 29 ♂ T. v.; 1/28, 27 hours after first treatment and again on 1/29 and 1/30, eggs were pressed from cloacal opening; 1/31, at autopsy, ovaries were still full of eggs, 13 eggs were in body cavity, 6 in right oviduct and 11 in the bladder.

No. 9. 1/31/38, ♀, wt 128.2 g, received 8.59 mg whole pituitaries from 53 T. v. (25 ♀ and 28 ♂ *or* 26 ♀ and 27 ♂); 2/2, 40 hours later, uteri seemed turgid and eggs were pressed from cloacal opening; 4.91 mg whole pituitaries from 16 ♀ and 14 ♂ T. v. were injected; 2/3, ♂ which had received 2.0 mg P.A. from 2 ♀ R. p. was placed with ♀; 2/4, ♂ attempted to clasp ♀ but unsuccessfully; 2/5, another ♂ (previously mated with frog *No. 4*) was placed with this ♀ but no clasping followed; 2/7, at autopsy, ovaries were still full of eggs, but 2 and a small bunch of cystic ones were found in the body cavity and 2 in left oviduct. Uteri were empty.

No. 10. 1/31/38, ♀, wt 117.4 g, received 5.07 mg whole pituitaries from 18 ♀ and 12 ♂ T. v.; 2/1, 3.56 mg, from 13 ♀ and 11 ♂ T. v. injected; 2/2, 5.38 mg from 17 ♀ and 19 ♂ T. v. injected; 2/1 and 2/2, no eggs expressed from cloaca by stripping; 2/3, ♂ which had received 2.57 mg P.A. from 2 ♀ R. p. was placed with ♀; 2/4, 18 hours later, ♂ was clasping ♀ and continued to do so during the day. In evening, large mass of eggs had been laid; 2/7, at autopsy, approximately 50 to 60 mature eggs were found in one lobe of the right ovary, 4 in the left, 6 in body cavity and 3 in the oviducts. Uteri were empty.

From the above data, it is evident that each of the 4 *Rana pipiens* females given homoplastic pars anterior, ovulated and in 2 cases egg-laying occurred. Among the 6 given whole pituitaries from *Triturus viridescens* in doses in which the anterior pituitary substance was approximately equivalent to that in doses of frog pars anterior administered to other females on the same date, 2 *(Nos. 6, 10)* gave excellent reactions, the ovaries being almost empty at autopsy, 3 *(Nos. 5, 8, 9)* responded by a slight amount of ovulation, and one *(No. 7)* failed to ovulate. It is, therefore, apparent that pituitaries of *Triturus viridescens,* a urodele, are able to induce ovulation in the anuran, *Rana pipiens,* when given in sufficiently large doses, but the degree of the reaction is variable. Previous experiments[8] have shown that *Triturus viridescens* will ovulate and lay eggs following implants of pars anterior or whole pituitary from *Rana pipiens.* Therefore, between these two amphibian forms there appears to be no "zoölogical specificity" of the gonad-stimulating hormones of the pars anterior.

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[8] Adams, A. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 433.

9932 P

Action of Colchicine on a Malignant Lymphoid Neoplasm in Mice of an Inbred Strain.

F. J. Lits,* A. Kirschbaum† and L. C. Strong.‡ (Introduced by Edgar Allen.)

From the Department of Anatomy, Yale University School of Medicine, New Haven.

In the normal animal lymphoid tissues have been found to be more susceptible than other tissues to the action of colchicine.[1, 2] Waves of mitoses and pyknoses are found in the thymus, spleen, lymph nodes and Peyer's patches following its administration in proper doses (Dustin's "caryoclasic shock"). The action of colchicine and certain other nuclear poisons is similar to that of X and gamma radiations, which led Dustin to refer to their effect as "radiomimetic". It thus seemed quite logical to test the action of this drug on a lymphoid tumor since (1) normal lymphoid tissue is so extremely sensitive to caryoclasic phenomena and (2) since other investigators were able to obtain regression of other types of tumors using this alkaloid.[3-7]

Mice of the C3H strain were used in this investigation. After a line of leukemia had been carried for 3 transplant generations experiments with colchicine were begun.§ When a piece of leukemic lymphoid tissue was implanted in the right axilla a large local growth formed previous to the appearance of systemic leukemia. The systemic disease did not appear until after 23 days' growth of the transplanted tumor. By 14 days the growth was the size of a kidney bean. If, at this time, 1/40 mg of colchicine in distilled water was administered subcutaneously every third day at a site far removed from the tumor the local growth practically disappeared. Average survival time of controls after transplantation was 31.5 days as compared with 50.5 days survival in treated animals (see Table I—14 test animals and 13 controls).** Life was significantly prolonged in 7 of the 14 treated mice (6 out of 8 where the drug was administered in 0.1 cc rather than 0.5 cc distilled water—see table). In no case was there complete regression of the tumor without recurrence. One treated mouse, however, survived 101 days after transplantation in contrast to the longest survival time of 42 days in the present control group (of 33 mice which have died following implantation of a few milligrams of this tumor, the longest survival time was 43 days).

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* C.R.B. Fellow in Anatomy and Surgery.

† Alexander Brown Coxe Fellow in Anatomy.

‡ Aided by grants from the International Cancer Research Foundation and the Anna Fuller Fund.

[1] Lits, F. J., *C. R. Soc. Biol.*, 1934, **115**, 1421.

[2] Lits, F. J., *Arch. Internat. de Méd. Exp.*, 1936, **11**, 810.

[3] Dominici, A., in *Thérapeutique Médicale*, Peau, Syphilis, Cancer, 1932, Paris, Ed. Masson.

[4] Amoroso, C., *Nature*, 1935, **135**, 266.

[5] Peyron, A., Lafay, B., and Kobozieff, N., *Bull. Assn. Fçaise pour l'Etude du Cancer*, 1936, **25**, 874

[6] Peyron, A., Poumeau-Delille, G., and Lafay, B., *Ibid.*, 1937, **26**, 625.

[7] Havas, L., *Ibid.*, 1937, **26**, 634.

§ Dr. W. U. Gardner[8] very kindly provided the authors with this material. The spontaneous leukemia arose in an animal which had received 3.9 mg of equilin benzoate in 303 days.

[8] Gardner, W. U., *Occasional Publications of the A.A.A.S.*, No. 4, 1937.

TABLE I.
Data on Survival Time.
Survival time in days after transplantation in control and colchicine-treated animals.

Controls	Colchicine	Transfer generation	Age of tumor when transplanted
1/40 mg colchicine administered in .5 cc distilled water.			
40	40	T_4*	23
42	101 (k)		
24	21	T_5	22
32	28		
	34		
	34		
1/40 mg colchicine administered in .1 cc distilled water.			
25	57	T_6	22
28	66 (k)		
33	68 (k)		
36			
26	44	T_7	19
30	45		
30	56		
31	56		
32	57		

(k)—killed for histological examination.
*T—transfer generation.

Histological studies revealed that following a third injection almost all the lymphoid cells of the tumor became pyknotic and died, the reticular cells of the lymphosarcomatous growth remaining (19 animals of the 9th transfer generation were used for the histological

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** 109 mice have been inoculated with this tumor in 14 transplant generations. There have been "takes" in all cases and a spontaneous regression has never been observed.

observations). Although islets of resistant lymphoid cells were found, regeneration of the tumor apparently began with proliferation of the stroma (reticular) cells. Non-leukemic spleen and lymph nodes of the test animals were histologically normal 72 hours after the third injection of colchicine.

Summarizing, colchicine therapy in a transplanted malignant lymphoid tumor lengthened survival time appreciably in 7 of 14 animals, but did not permanently suppress tumor growth. The reticular stroma cells of the tumor were most resistant to therapy and probably are "malignant" elements in the lymphoid growths of mouse leukemia. "Malignant" lymphocytes proved to be more susceptible to colchicine action than normal splenic and lymph node lymphocytes in the treated tumor-bearing animals. Thymic lymphocytes were, however, killed by colchicine treatment. It is emphasized that colchicine administration is in no sense considered a cure for malignant lymphoid growths, but a drug which may be used to advantage in the experimental histological investigation of such tumors. So far colchicine has been found to hasten death in those animals where lymphoid neoplastic disease was systemic.

9933 P

Quantitative Separation and Estimation of Various Porphyrins in Biological Materials.

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We extract the porphyrins from urine, bile and feces by prolonged extraction with acetic acid-ether (Fink and Hoerburger,[1] Fikentscher,[2] Van den Bergh and others,[3] Brugsch[4]). Uroporphyrin is lost but, according to Fischer and Zerweck,[5] this may be neglected except in extremely rare cases. The porphyrins are taken up by 5% HCl from the crude ether extract. Purification involves repetition of the transfer to ether and back to HCl. Phylloerythrin requires 10% HCl. Total porphyrin is estimated in 5% HCl by the intensity of the red fluorescence excited by ultraviolet light. Pure copro- or hematoporphyrin solution is used for standardization. Recovery of known amounts of pure porphyrins in 12 series of determinations averaged: coproporphyrin, 100%; hemato-porphyrin, 104%; mesoporphyrin, 97%; protoporphyrin, 103%.

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[1] Fink, H., and Hoerburger, W., *Z. physiol. Chem.*, 1931, **202**, 8.

[2] Fikentscher, R., *Biochem. Z.*, 1932, **249**, 257.

[3] Van den Bergh, A. A. H., Grotepass, W., and Revers, F. E., *Klin. Wschr.*, 1932, **11**, 1534.

[4] Brugsch, J. T., *Z. ges. exp. Med.*, 1935, **95**, 471.

[5] Fischer, H., and Zerweck, W., *Z. physiol. Chem.*, 1924, **137**, 242.

Quantitative fractionation of the porphyrins is done by successive extractions of the purified ethereal solution with 1 volume 0.25% HCl to 3 volumes ether. Three such extractions remove all the coproporphyrin (and hematoporphyrin which, however, does not occur naturally), 62% of the mesoporphyrin, 10% of the protoporphyrin, and about 49% of the deuteroporphyrin.

These results on pure porphyrins apply also to mixtures. Quantitative estimates of copro- and protoporphyrin in known mixtures are precise within ±3% of the total; similar mixtures of copro- or proto- with mesoporphyrin were separated with an error no greater than ±6%.

With the foregoing mixtures, and with biological materials which can be treated as 2 component mixtures of porphyrins, the calculation is:

(1) Total porphyrin $= A + B$.

(2) $B = M/(1-y)^n$, where n is the number of extractions required to remove A completely, M is the amount of porphyrin remaining after n extractions and y is the distribution coefficient in extraction number $n + 1$.

(3) $y = r_n/M$, where r_n is the amount of porphyrin extracted in extraction number $n + 1$.

There is rarely any important error in treating urine and bile as 2-component systems of copro- and non-coproporphyrin; feces may also be so treated but in some cases the error may exceed 10% of the coproporphyrin. Results are satisfactory with 5 to 200 micrograms of total porphyrins.

Five hundred analyses of urines from 142 normal individuals show that the 24-hour output of normals never exceeds 100 gammas of porphyrin, and that less than 10% are over 60 gammas. At least 95% of the urine porphyrin is normally of the coproporphyrin type.

In human bile 60 to 85% of the porphyrin is of the coproporphyrin type. We did not find phylloerythrin in human bile. In ox bile we found only 10 to 20% coproporphyrin; about 30% was phylloerythrin and nearly half of the total porphyrin was of the pyrroporphyrin and other intermediate types.

In human feces with either negative or questionable benzidine reaction, coproporphyrin type accounted for, on the average, 30 to 40% of the porphyrins. Feces with a positive benzidine reaction (free hemin) averaged only 18% coproporphyrin type, reflecting a great increase in protoporphyrin. Acholic feces (biliary obstruction) showed zero to 3% coproporphyrin in the patients studied.

In all feces we found a considerable amount of porphyrin of the meso type similar to Schumm's[6] "sapro-porphyrin D." We also find in human feces very minute amounts of a chloroform-soluble porphyrin with a rather low HCl number; this may be what Watson[7] has termed "pseudo-deutero porphyrin."

Quantitative separation and analysis of porphyrins in the feces seems to offer a sensitive means for early detection of biliary ob-

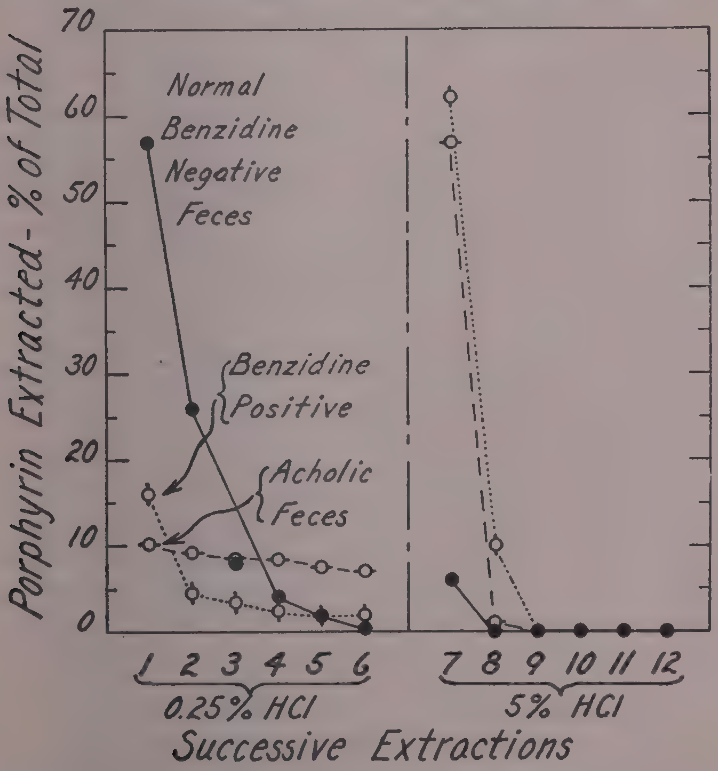

FIG. 1.

Extraction curves for porphyrins in human feces. In each extraction 1 volume of acid was used for 3 volumes of the ethereal solution.

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[6] Schumm, O., *Z. physiol. Chem.*, 1927, **169**, 3, 52.

[7] Watson, C. J., *J. Clin. Invest.*, 1937, **16**, 383.

struction and perhaps for the occurrence of bleeding in the gastrointestinal tract. Typical extraction curves from human feces are shown in Fig. 1.

9934 P

Blood Potassium Changes as a Result of Partial Asphyxia in Dogs.

JOE DENNIS AND F. J. MULLIN.

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This work grew out of previous studies showing that ischemia of the functioning heart leads to a marked rise in the potassium content of the coronary venous blood.[1] This report is concerned primarily with the effects produced in anesthetized dogs on clamping off the tracheal cannula for periods of from 2 to 4 minutes. Twelve dogs anesthetized with nembutal were used. In half the dogs only one asphyxial period was produced, in the others 2 or more were obtained. Blood was drawn from the external jugular vein in 10 of the dogs. In 2 dogs the chest was opened and artificial respiration administered, the blood being obtained from the superior cava in one and from the right auricle in the other. The method of Breh and Gaebler[2] was used in the potassium determinations, the analysis being completed by the diazotization procedure of Briggs.[3] The condition of the blood samples was unknown to the analyst, since they were given blind numbers when they were drawn.

The results are given in Table I. They show that during asphyxia the plasma potassium increases 22% and the whole blood potassium 16% over the normal control values. There is an increase of 43% for the plasma and 14% for the whole blood potassium during a second asphyxial period, as compared to its control taken immediately before. The second asphyxial period was produced from 10 to 20 minutes after the first one.

These figures show the increase in blood potassium during asphyxia. Data on the effects of over-ventilation are as yet inconclusive. Fenn has shown that muscular activity causes a decrease in

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[1] Dennis, Joe, and Moore, R. M., *Am. J. Physiol.*, in press.

[2] Breh, F., and Gaebler, O. H., *J. Biol. Chem.*, 1930, **87**, 81.

[3] Briggs, A. P., *J. Biol. Chem.*, 1923, **57**, 351.

TABLE I.
Blood Potassium Changes in Asphyxia, Expressed as Mg.%.

Dog No.	Normal Control		At End of Asphyxial Period		Control After Recovery from Asphyxia		Further Asphyxial Period	
	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood
1	21.5	30.8	23.4	31.9	22.5	36.5	31.3	43.2
2			21.6		9.2		21.4	
3	19.5		22.5					
4	15.8		20.6					
5	20.6		19.0					
6	21.7		33.2	35.0	18.9	29.9	25.7	34.8
7	17.4	24.1	18.7	25.0				
8	20.6	23.9	22.7	29.0				
9	12.4	19.2	18.3	21.9	12.3*			
10	19.4	23.0	26.3	27.8				
11	15.5		19.8		16.9*			
12	15.2	22.6	19.9	24.8	16.1	26.9	17.2	28.1
Avg	18.1	23.9	22.2	27.9	16.0	31.1	23.9	35.4

*Not used in computing changes caused by second asphyxial period.

muscle potassium.[4] Presumably this would be accompanied by an increase in blood potassium. Producing tetany in the anesthetized dog by reducing the cerebrospinal fluid calcium ions or increasing the potassium ions in the cerebrospinal fluid,[5] has caused a rise in blood potassium in some experiments we have recently performed. We have reason to believe that the increased potassium ions do not escape from the cerebrospinal fluid into the blood under the conditions of our experimental procedure. Furthermore, even if all the potassium injected into the cisterna magna passed immediately into the blood stream, it would not be sufficient to account for the increase in potassium in the blood that we observed. Seemingly, asphyxial conditions or muscular tetany cause a release of potassium into the blood stream.

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[4] Fenn, W. O., *Am. J. Physiol.*, 1937, **120**, 675.

[5] Mullin, F. J., Hastings, A. B., and Lees, W. M., *Am. J. Physiol.*, 1938, **121**, 719.

9935 P

Comparative Estimation of Blood Supply of Skin Areas from Photoelectrically Recorded Volume Pulse.

Alrick B. Hertzman.

From the Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

The principle of the photoelectric plethysmograph[1] has been applied to the comparison of the richness of the blood supply in several skin areas. Vertical illumination of the skin is provided by an ophthalmoscope bulb. Enough light is scattered through the subcutaneum to provide a sufficiently satisfactory transillumination to permit the detection of changes in the circulation in the skin by a photoelectric cell placed over the transilluminated area. The variations in the photoelectric current with changes in the blood content of the skin are recorded on the electrocardiograph after amplification.

The volume pulse of the skin so recorded probably is an index of the arterial inflow with each heart beat and so can be used as an index of the blood supply of that skin area. (Special hemodynamic conditions will, of course, limit the specific application of the statement). Arterial constriction in the skin decreases the volume pulse; vasodilatation increases it. The amplitude and form of the recorded wave will also vary with the size and number of arterial vessels in the transilluminated area.

Quantitation of the recorded wave in terms of arbitrary units is effected by comparing the size of the wave with the deflection obtained from inserting a glass filter (a thin glass sheet) between the photoelectric cell and the transilluminated skin area. Although the question as to what is the actual blood equivalent of the filter in terms of cc of blood per 100 cc of skin tissue is an involved one,[1] a tentative approach to the problem is provided in the data of Turner, *et al.*[2] Their average volume pulse in the finger tip at heart level is 2.8 mm^3 or .06% of the soft tissue volume. Calling this value equivalent to 1.85 filter units (which is the average amplitude of the photoelectrically recorded volume pulse in the finger tip also at heart level), one obtains by substitution of the blood equivalent of the filter, the values for the volume pulse in the different skin areas as recorded in Table I. These values were obtained on 6 physically vigorous medical students, in the sitting position, comfortably clothed, after 20 minutes of rest. (Room temperature usually 80°F; relative humidity 30.) The values showed considerable variation in the different individuals although the order of amplitude of the volume pulse in the different skin areas was essentially the same in the different subjects. Simultaneous skin temperature measurements showed a general correlation with the amplitude of the volume pulse, although marked exceptions appeared if the skin were moist. Wrapping the subject in heavy blankets and making the observations on the warm covered skin gave notable increases in the amplitude of the volume pulse of the skin but even so it was not possible thus to obtain waves in the warm covered skin of the forearm and thigh more than 40% as large as those in the exposed skin of the forehead. It is, therefore, tentatively concluded that the figures in the table represent the approximate order of differences in the richness of the arterial supply to the corresponding skin areas.

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[1] Hertzman, A. B., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 290, 529.

[2] Turner, R. H., Burch, G. E., and Sodeman, W. A., *J. Clin. Invest.*, 1937, **16**, 789.

The high values for the finger tips correlate with the abundance of small arteries here. Skin vessel counts are needed to extend such correlations. It is interesting to note that the areas (finger and face) which show the largest volume pulse values are also those in

TABLE I.
Amplitude of the Volume Pulse in Several Skin Areas.

Skin Area	Amplitude of Volume Pulse in Filter Units	Calculated Amplitude Vol. Pulse cc/100 cc Tissue
Finger tip (palmar)	1.85	.06
Thumb tip ”	1.85	.06
Lip	1.16	.038
Nose	1.07	.035
Hypothenar eminence	1.04	.034
Forehead	1.00	.032
Ear	0.97	.031
Thenar eminence	0.90	.029
Palm of hand	0.90	.029
Cheek	0.68	.022
Big Toe (plantar)	0.66	.021
Finger (dorsum)	0.54	.017
2nd toe (plantar)	0.46	.015
Hand (dorsum)	0.37	.012
Neck (dorsum)	0.34	.011
Foot (plantar)	0.25	.008
Knee	0.20	.006
Forearm	0.17	.005
Big toe (dorsum)	0.15	.005
2nd ” ”	0.15	.005
Foot ”	0.11	.003
Tibia, thigh, arm	indeterminate	

which vasomotor reactions seem to be most marked. Studies are in progress to determine the maximum circulation which can be attained in the various skin areas, as well as to examine the effects of peripheral vascular disease on the blood supply of these areas.

9936 P

Studies on the Effects of the Administration of Insulin by Iontophoresis.

LAY MARTIN. (With the technical assistance of Hubert Ruland.)

From the Gastro-enterological Division, Department of Medicine, Johns Hopkins University and Hospital

We have been able to show that by iontophoresis a large molecule such as acetyl beta methylcholine could be introduced through the intact skin and be absorbed into the blood stream.[1] Acetyl beta methylcholine bears a positive charge and was electrolysed at the anode. Insulin bears a negative charge, for experimental purposes the effects of electrolysis at each pole was attempted.

Dogs were used as the experimental animal. They were in an 18-hour postprandial state when the experiments were performed. The hair from the abdomen and one thigh was carefully shaved. The animal was restrained in a supine position and a folded piece of asbestos paper, saturated with insulin U-80 was placed on the bare abdominal skin. It was covered by a piece of block tin of sufficient size to fit within the limits of the paper. The other pole was placed on the thigh and held in place by means of a cotton bandage.

The instrument used was a simple but effective one consisting of a 45-volt B battery with rheostat control and an attached ammeter capable of measuring milliamperes. The circuit was closed and the amperage was slowly raised to 30 milliamperes and kept there 30 to 60 minutes as indicated in Table I. Capillary blood sugar estimations were made by the Folin micro method.[2] The control level was determined just before the circuit was closed. Subsequent estimations were made after the circuit had been broken for 30, and for 60 minutes, and then at hourly intervals for 6 hours.

Two control tests were made with distilled water. In one instance it was placed beneath the cathode and in the other anode.

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[1] Martin, L., *New England J. of Med.*, 1937, **207**, 202.

[2] Folin, Otto, and Malmros, Haqvin, *J. Biol. Chem.*, 1929, **83**, 115.

TABLE I.

Date	Polarity	Material used	Control	Treatment hr	Blood sugar end of treatment	½ hr after treatment	1 hr after treatment	2 hr after treatment	3 hr after treatment	4 hr after treatment	5 hr after treatment	6 hr after treatment	Remarks
4-13	+	Insulin	70	½	74	71	83.5	71.5	73	65.8	75.8		
4-20	—	,,	96.6	½	95.2	89	86	84.4	77	78.5	80		
4-28	—	,,	88.6	1	89	82	86	86	83	77.2	76		
5-5	—	,,	78	1	73	52.6	54	62.7	63.5	62.5	71.8	68.6	
5-12	—	,,	59	1	43	33.3	36.6	46.5	43.8	54	51.8	58.3	Considerable Shivering
5-19	—	,,	81.6	1	74.1	77	75.1	70.1	72.7	68	70.8	66.6	Bad Burn
5-26	—	,,	107	1	96.7	91.3	89	89	80.6	80	74.4	78.6	,, ,,
6-2	+	Distilled water	77	1	77	70.5	78.3	84.9	77	78.5	80	84.9	
6-16	—	,, ,,	89	1	91.5	92	91.5	102	91	92.6	87		

No significant change in blood sugar level was noted. In the one instance when the insulin was placed beneath the positive pole there was no important change in blood sugar level.

On 6 occasions insulin was placed beneath the negative pole and in each experiment there was a drop in blood sugar. The smallest fall was 12 mg per 100 cc; the largest 32 mg per 100 cc. In one dog the level of blood sugar dropped from 59 mg per 100 cc to 33 mg per 100 cc and at this period the animal showed signs of an hypoglycemic reaction.

It was not possible to predict when the sugar would fall. At times it occurred within an hour or two after the termination of iontophoresis. In other experiments it occurred later.

The thought naturally occurs as to the propriety or advisability of using such a form of administration in the treatment of diabetes in man. The data we have obtained do not permit us to draw any conclusions. However, it might be pointed out, as we have noted not only in these experiments but in those in which acetyl beta methylcholine was used in man, that there is apparently considerable variation in skin resistance in different individuals. It is conceivable that a technic might be developed which could control the variable skin resistance and deliver a known amount of insulin to the body. Such a method would offer the particularly desirable condition of providing a slowly absorbable supply of insulin.

Summary. A fall in blood sugar in dogs has been demonstrated following cathode iontophoresis with insulin.

9937 P

Physiological Effects of Some Thio-Esters of Choline.

W. F. Alexander, J. B. Dillon and C. N. Jordan. (Introduced by Albert Kuntz.)

From St. Louis University School of Medicine.

The effects on blood pressure and respiration produced in cats and dogs under morphine-urethane, nembutal and chloralose anesthesias, by intravenous injections of thio-acetyl choline chloride, thio-acetyl-gamma-homo-choline chloride and thio-acetyl-beta-methyl choline chloride have been observed and recorded.* Eighteen animals (10 cats and 8 dogs) have been used in these experiments. Records were taken of the results of over 200 injections, of which approximately one-third was of each of the 3 compounds. The results of the administration of each compound were constant.

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* The chemistry of these compounds will be reported separately.

Thio-acetyl-choline chloride and thio-acetyl-gamma-homo choline chloride are almost identical in their effects. Immediately following injection the blood pressure falls slightly, then rises rapidly to 60 to 90 mm Hg above the normal level. During this rapid rise the heart rate increases but upon reaching the peak pressure the rate slows and large vagal beats appear. The duration of this bradycardia varies from one to 5 minutes depending upon the amount of the compound injected. During this period the blood pressure gradually falls to normal, which level is reached in 2 to 10 minutes. Hyperpnea of short duration immediately follows injection. This hyperpnea is followed by depression with gradual return to normal.

Thio-acetyl-beta-methyl choline chloride produces a moderate depressor action but has no effect upon the respiration. B.P. falls 30-50 mm Hg and is not accompanied by changes in heart rate.

The minimum effective dose is of the order of .016 mg/kg. When larger dosages are used (.5-1 mg/kg) the animals rapidly pass into a fatal apnea.

Atropine sulphate abolishes neither the pressor response nor the hyperpnea caused by thio-acetyl choline chloride and thio-acetyl-gamma-homo choline chloride, but eliminates the bradycardia. The depressor action of thio-acetyl-beta-methyl choline chloride is abolished by atropine. Physostigmine does not augment the action of any of these compounds. Section of the vago-sympathetic trunk alone does not eliminate the pressor responses to thio-acetyl choline chloride and thio-acetyl-gamma-homo choline chloride but diminishes the hyperpnea. Denervation of the carotid sinuses alone does not affect the hyperpnea of thio-acetyl choline chloride or of thio-acetyl-gamma-homo choline chloride. No carotid sinus denervation has been carried out with thio-acetyl-beta-methyl choline chloride.

After denervation of the carotid sinus and section of the vago-sympathetic trunk in the neck, thio-acetyl choline chloride and thio-acetyl gamma-homo choline chloride have no effect upon respiration but still produce their pressor effect which is preceded by a slightly augmented depressor response.

The duration of the action of these compounds indicates that they are probably destroyed rather rapidly by the blood. Thio-acetyl-beta-methyl choline chloride is very evanescent in its action and is probably hydrolyzed more rapidly than the other two thio-esters.

When thio-acetyl choline is placed in freshly drawn blood and allowed to stand at room temperature for 30 minutes to one hour, its action is greatly reduced and in some cases reversed.

The results suggest that thio-acetyl choline chloride and thio-acetyl-gamma-homo choline chloride have a nicotine-like action; however, when respiratory failure occurs, stimulation of the phrenic nerve still causes tetanic contraction of the diaphragm. Thio-acetyl-beta-methyl choline chloride has a muscarine-like action; however, its action is not augmented by physostigmine.

9938 P

Attenuation of Toxins by Interfacial Adsorption.*

J. M. JOHLIN.

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The writer has previously demonstrated that crystalline insulin[1] can be attenuated by interfacial adsorption. In the same communication reference was made to the fact that bacterial toxins can also be attenuated by a similar procedure. It has been found by additional experimentation that ricin, tetanus toxin, staphylococcus toxin and snake venom may be completely detoxified by this method and still retain antigenic properties adequate for purposes of immunization, as demonstrated with rabbits and mice. The following data are representative of the results which have been obtained.

"C.P. ricin" which will produce a pronounced erythema, induration and necrosis when 0.2 cc of a solution of 1 part of ricin in 25,000,000 parts of saline are injected intradermally into rabbits will produce only a faint erythema when equal volumes of the same ricin in dilutions of 1 part of ricin in 1,000 parts of saline are injected after 2 treatments of 48 hours each with chloroform. When triple injections of untreated ricin in dilutions of 1 part ricin in 1,000,000 parts of saline were injected intradermally into rabbits which had been immunized with completely detoxified ricin, only a faint erythema was produced at the site of each injection. When

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* Aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation and by a grant from the American Medical Association.

[1] Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 523.

the serum of each immunized rabbit was mixed with an equal volume of a solution of ricin containing 1 part of ricin in 200,000 parts of saline, and the mixture (0.2 cc) injected intradermally into normal rabbits, no symptoms of any kind were produced.

Tetanus toxin† containing 70,000 (guinea pig) M.L.D.'s per cc was completely detoxified when treated twice for 48 hours by interfacial adsorption. Five cc of the detoxified toxin produced no symptoms of any kind when injected subcutaneously into guinea pigs. Groups of mice (50 each) treated with the detoxified toxin all survived this treatment and smaller groups of these subsequently survived injections of 1 and 2 M.L.D.'s of untreated toxin without symptoms of tetany. A smaller proportion of other groups survived injections of 3 to 15 M.L.D.'s of untreated toxin with symptoms of tetany.

Solutions of staphylococcus toxin† "containing 8,000 dermonecrotic units and 700 hemolytic units per cc" was completely detoxified by 2 treatments of 48 hours each. Some of the solution, in which all hemolytic, lethal and skin necrotizing action had thus been eliminated by adsorption, was injected into rabbits and increased the protection of these animals towards staphylococcus toxin about 20 times.‡

The toxic and dermonecrotic action of 0.2% solutions of moccasin venom was entirely eliminated after treatment for a 36-hour period. In groups of mice immunized with the treated toxin, as many as 13 out of 15 survived subcutaneous injections of 0.1 cc of a 1% solution of the untreated toxin, while 2 out of 3 of each of 3 groups of controls died when injected with 1/4, 1/2, and 1/1 times this amount.

Since toxins, on standing for longer periods of time, are spontaneously converted into toxoids, the effect produced by surface adsorption is undoubtedly that of the catalytic action of surface concentration and orientation. Further tests are being made with virus suspensions. The results indicate that this method of attenuation might be generally applied to biological products of a protein-like nature.

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† Obtained through the courtesy of the Lederle Laboratories.

‡ Experiments with staphylococcus toxin were performed conjointly with Dr. R. H. Rigdon.

9939

A Note on the *Beta* Hemolytic Streptococci of Air.*

L. Buchbinder, M. Solotorovsky and M. Solowey. (Introduced by Beatrice C. Seegal.)

From the DeLamar Institute of Public Health, College of Physicians and Surgeons, Columbia University, New York City.

In a routine study of the air of such places as schools, theaters, the subway and streets in New York City *alpha* hemolytic streptococci were regularly found. Many strains were isolated which presumably originated in the human nasopharynx.[1] In contrast the occurrence of *beta* hemolytic streptococci was relatively infrequent. However 52 strains were isolated, all but 5 from indoor locations.† The properties of these strains were compared with those of known human pathogenic strains. The following tests were applied (1) Antigenic grouping (Lancefield[2]). (2) Lysis of human fibrin (Tillet and Garner[3]). (3) Hemolysis in poured sheep and rabbit blood-agar plates. (4) Final pH in 1% dextrose broth after incubation for 7 days. (5) Fermentation of trehalose, sorbitol, lactose, salicin, and mannite. (6) Hydrolysis of sodium hippurate. (7) Reduction of methylene-blue milk.

Forty-six of the 52 strains belonged to Lancefield's group A; 4 of these which were isolated from street air were indistinguishable from those obtained from indoor air. One strain belonged to group B, 3 to group C, 1 each to groups D and G. None of the strains reacted with group F or H serums.

The group A strains isolated from air were compared, for lysis of human fibrin, hemolysis of sheep and rabbit blood and final pH produced after 7 days' growth in dextrose broth, with 44 strains of group A streptococci isolated from human sources.‡ Both groups of strains were isolated during the same time period. It was found that 37 of 45 air strains lysed human fibrin within one hour, 28 of these within 20 minutes; while 3 produced complete lysis overnight,

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* Supported by a grant from the John B. Pierce Foundation.

[1] Buchbinder, L., Solowey, M., and Solotorovsky, M., *Am. J. Pub. Health*, 1938, **28**, 61.

† The cultures were collected by the Air Pollution Survey sponsored by the Department of Health of New York City.

[2] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

[3] Tillet, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

‡ We are indebted to Dr. John Lytle, Dr. David Seegal, and Miss Elizabeth Jost for these cultures.

1 lysed fibrin only partially and 4 failed to lyse in 2 tests. Of 44 strains from human sources 35 lysed fibrin completely in 20 minutes, 8 in 80 minutes and one overnight. Thus it seems that on the whole the fibrinolytic function was slightly if at all affected by aërial environment. The hemolysis of sheep and rabbits' blood in agar plates by the air and human strains was practically indistinguishable. The range of final pH in dextrose broth was also the same in both groups, that is 4.6 to 5.2.

All the group A air strains fermented trehalose, but failed to ferment sorbitol, or to reduce methylene-blue milk or to hydrolyze sodium hippurate. These are the accepted reactions of group A strains from human sources. All but 9 strains fermented lactose, all but 5 fermented salicin, while only 5 strains fermented mannite.

The 3 group C strains apparently were of the so-called "human" type since they all fermented trehalose but not sorbitol, and lysed human fibrin.[4] The one group B strain hydrolyzed sodium hippurate, fermented trehalose but not sorbitol, and failed to lyse human fibrin. The group D strain was apparently one of the fecal streptococci. It fermented trehalose, salicin, lactose, and mannite, reduced methylene-blue milk, produced a final pH of 4.3 in glucose broth, and failed to hydrolyze sodium hippurate, to lyse human fibrin or to ferment sorbitol. The group G strain fermented lactose and failed to attack any of the other substances tested.

Discussion and Summary. Although it has been shown recently that the air of hospital wards contains *beta* hemolytic streptococci of the same serological types as those infecting patients in the same wards[5,6] it should be emphasized that the strains reported on here came from air breathed by the city population at large. Though our findings indicate that group A streptococci are relatively rare in normal air sampled at random, it is of great interest that, by the tests applied, they are indistinguishable from similar organisms isolated directly from disease processes. Furthermore, it should be noted that 88% of the strains in this small series were of the human pathogenic group A.

It has been estimated by several investigators[7, 8, 9] that group A hemolytic streptococci are present in from 3 to 7% of normal throats. None have reported as high a percentage of group A cultures among the hemolytic streptococci of the throat as was found in this series of air streptococci. It is possible that the percentage of group A cultures of air was augmented by the coughing of individuals with group A infections of the throat.

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[4] Sherman, J. M., *Bacteriol. Rev.*, 1937, **1**, 1.

[5] White, E., *Lancet*, 1936, **1**, 941.

[6] Brown, W. A., and Allison, V. D., *J. Hyg.*, 1935, **37**, 1.

[7] Hare, R., *J. Path. and Bact.*, 1935, **41**, 499.

[8] Davis, L. J., and Guzdar, J. S., *J. Path. and Bact.*, 1936, **43**, 197.

[9] Frisch, A. W., *J. Inf. Dis.*, 1938, **62**, 40.

The presence of group A streptococci does not necessarily indicate that they were suspended in droplets in air, for, as shown by studies of hospital air[5, 6] and as has been demonstrated experimentally by Wells and in this laboratory, bacteria sprayed in air may float for many hours after all droplets have evaporated. In conclusion we agree with Brown and Allison who, after studying the streptococci in the air of scarlet-fever wards, stated, " . . . . and while contact, direct or indirect, is probably of considerable importance in the transmission of infection the possibility of infection via the air other than that due to droplets cannot be dismissed."

Thanks are due to Dr. Beatrice C. Seegal for her interest and assistance as well as to Dr. Rebecca C. Lancefield for a generous contribution of group serums.

9940 P

Germicidal Efficiency of Some Silver Compounds Tested by the Improved Tissue-Culture Method.

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Silver compounds are employed for their antiseptic and germicidal action on bacteria. The effectiveness of such preparations is due largely to the free silver ions. The higher the concentration of free ions the greater will be the germicidal effect. *New and Nonofficial Remedies* (1937) states, "The antiseptic action of silver nitrate is complicated by irritation, pain, astringency, and corrosion. These may be desirable for the destruction of tissue or the stimulation of indolent wounds; but when they are not necessary for such purposes, they are distinctly undesirable. They may be avoided by the use of colloidal silver preparations."

The colloidal preparations differ from the silver compounds in that the silver does not exist to any great extent in the form of free ions. The silver does not, therefore, precipitate chlorides or proteins and is noncorrosive and relatively nonastringent and nonirritating. The germicidal action is not proportional to the silver content of the preparations but is due to the liberation of very low concentrations of silver ions. The degree of ionization or liberation of silver ions varies with different preparations.

In an earlier paper[1] a new method was proposed for the evaluation of germicidal substances. The compounds were tested for their effect on the growth of living embryonic chick tissue as well as for their ability to kill bacteria. A number known as the toxicity-index was determined which is defined as the ratio of the highest dilution of disinfectant showing no growth of embryonic tissue in 10 minutes to the highest dilution required to kill the test organism in the same period of time. The tests were run at a temperature of 37°C in the presence of a standard amount of organic matter. Theoretically the smaller the index the more nearly perfect the germicide.

In the present paper a number of silver preparations were tested. These were: (1) silver nitrate; (2) silver lactate; (3) silver citrate; (4) silver protein mild, U. S. P.; (5) silver protein strong, U. S. P.

Effect of germicides on bacteria and tissue. The silver preparations were tested against embryonic chick tissue and the organisms *Staphylococcus aureus* and *Eberthella typhosa*. The killing concentrations of the germicides for tissue and bacteria and their toxicity-indices are given in Table I.

TABLE I.

	Highest dilution showing no growth after 10 min. at 37°C in the presence of organic matter			Toxicity index = A/B	
Compound	*Staph. aureus* = B	*E. typhosa* = B	Tissue = A	*Staph. aureus*	*E. typhosa*
Silver lactate	1:110	1:690	1:100	0.9	0.15
” citrate	1:600	1:4500	1:610	1.0	0.14
” nitrate	1:80	1:1250	1:140	1.8	0.11
” protein strong, U.S.P.	1:15	1:175	1:25	1.7	0.14
” protein mild, U.S.P.	1:12*	1:70	1:30	2.5	0.43

*Failed to kill. A more concentrated solution could not be prepared.

The results show that of the non-colloidal compounds silver lactate and silver citrate are of about the same order of toxicity against *S. aureus*, being superior to silver nitrate. On the other hand the 3 compounds exhibit about the same degree of germicidal action against *E. typhosa*.

Of the colloidal organic compounds tested silver protein mild,

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[1] Salle, A. J., McOmie, W. A., Shechmeister, I. L., and Foord, D. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 694.

U. S. P., cannot be relied upon to destroy *S. aureus* in the presence of the concentration of organic matter used in the above experiments. However, it rated considerably better against *E. typhosa*. On the other hand, silver protein strong, U. S. P., was effective against both organisms but rated slightly lower than the non-colloidal compounds.

9941

Inefficacy of Prontylin in Experimental Tuberculosis.

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The interest in chemotherapy aroused by the discovery of the therapeutic value of prontosil and related compounds has been applied to the study of their effects against many infectious agents. At least one prior report has been concerned with the chemotherapy of tuberculosis with Prontylin. Rich and Follis[1] administered the drug as dry crystals by mouth to guinea pigs inoculated subcutaneously, and sacrificed both treated and control animals 5 or 6 weeks after inoculation. Pathological surveys revealed less extensive lesions in treated than in control animals and indicated a possible beneficial effect of the drug.

In our experiments we have employed the intracerebral route of inoculation,[2] by means of which acute disease and death are induced in a short time by a small number of virulent organisms. The criterion of efficacy was survival-time following such inoculation.

Twenty healthy male guinea pigs were divided into 2 groups of 10 according to weight. Individual weights of 9 animals in the 2 groups were identical, and the mean weights of the 2 groups were 344 and 350 g. The twenty animals were given intraäbdominal injections of Seconal,* 20 mg per kg, to induce anesthesia, and were then inoculated intracerebrally with 0.00001 mg (moist weight) of a human strain of tubercle bacilli (designated Lockett), isolated in this laboratory. Beginning 24 hours after inoculation 10 animals were given 200 mg of Prontylin† in 10 cc physiological saline daily by intraäbdominal injection. It was found that a solution of this strength could be prepared by heating the salt solution, dissolving the drug, and cooling to body-temperature. Injections were then made immediately before recrystallization took place. This dose was given daily for 23 days, at which time it was increased to 250 mg in 12.5 cc saline solution. The latter dose was given daily for 7 days, when treatment was discontinued because the animals were not benefitted by it.

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[1] Rich, A. R., and Follis, R. H., Jr., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 77.

[2] Smithburn, K. C., *J. Exp. Med.*, 1936, **64**, 771.

* Sodium propyl-methyl-carbinyl allyl barbiturate, Lilly, supplied by Eli Lilly and Company, through Dr. G. F. Kempf, Indianapolis City Hospital.

The first death in the control group occurred on the 24th day and the last on the 51st day following inoculation. In the treated group the first death occurred on the 12th and the last on the 49th day. The mean survival time for the 2 groups was 34.1 ± 1.9 and 30.8 ± 1.6 days respectively. This difference is not significant. Gross necropsy-findings in the 2 groups were remarkably similar.

The difference in the methods we have used and those referred to above[1] are fourfold. We have used fewer organisms and have inoculated intracerebrally rather than subcutaneously; we have given the Prontylin intraäbdominally in solution rather than by mouth; and lastly, we have relied upon survival-time to determine the effect. Since this drug is effective in certain other bacterial infections of the meninges, we do not consider that the route of inoculation invalidates this experiment. Moreover, survival-time after intracerebral inoculation of tubercle bacilli can be influenced by other means to be reported later. However, the possibility cannot be excluded that Prontylin may be more effective by mouth than by parenteral injection.

Conclusion. Under the conditions of our experiments, Prontylin exerted no beneficial effect in experimental tuberculosis.

9942 P

Effect of Age of the Host on Resistance to Tuberculous Infection.

KENNETH C. SMITHBURN.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

In order to standardize procedures for virulence-tests, or tests on immunization, it is necessary to know the possible sources of variation and to control them insofar as it is possible. The experiments to be discussed were done to ascertain whether or not young guinea pigs were more susceptible to experimental tuberculosis than older, mature animals.

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† *p*-amino-phenyl-sulfonamide, supplied by Winthrop Chemical Company, Inc.

Three strains of tubercle bacilli were used in the experiments: a bovine strain 39, isolated by the late Dr. Theobald Smith, and 2 lines of the human type strain H-37, one moderately virulent, the other highly virulent. Albino guinea pigs of both sexes were used but males were caged separately from females. All animals were inoculated intracerebrally[1] under anesthesia induced by the intraäbdominal injection of 0.5% solution of Seconal,* the dose being the equivalent of 20 mg per kg. All animals were allowed to succumb to the infection. A necropsy was performed on each animal at death in order to make pathological surveys and detect any possible intercurrent factors.

In the first experiment, 3 female guinea pigs weighing 840 to 1060 g and 4 immature males weighing from 120 to 180 g were inoculated intracerebrally with 0.001 mg of the bovine strain 39. In addition, one female and 2 male guinea pigs weighing from 680 to 860 g, 2 males and 2 females weighing 100 to 140 g were inoculated intracerebrally with 0.001 mg of the moderately virulent line of the human strain H-37. The mean survival for those receiving the bovine organisms was 21.5 days for the old and 27.25 days for the younger animals. Among those receiving the H-37 the average survival was 31.3 days for the old animals and 32.6 days for the young animals. Two animals in this experiment died from complicating non-tuberculous pneumonia and were excluded from consideration.

In a second experiment 3 females and 2 males weighing 860 to 1040 g constituted one group. Five male guinea pigs weighing 380 to 480 g constituted a second group, and 5 females weighing 100 to 160 g constituted a third group, so that the experiment included old, middle-aged, and young animals. Each animal of the 3 groups was inoculated intracerebrally with 0.00001 mg of the highly virulent line of human tubercle bacilli, strain H-37. All animals received the same dose of the same suspension of the organisms within the same hour. The mean survival for the old animals was 23.8 ± 0.80 days; for the middle-aged group, the mean was 27.6 ± 1.10 days, and for the young it was 35.8 ± 0.78 days. The differences were compared by statistical analysis and proved to be significant. The differences in mean survival of the old and middle-aged group, 3.8 days, is 2.8 times the probable error of that difference, and the odds against occurrence by chance are about 15 to 1. The difference in mean survival of the middle-aged and young groups, 8.2 days, is 6.1 times its probable error and the odds against chance occurrence are very high. More striking still, the difference in mean survival of the old and young groups, 12.0 days, is 10.8 times the probable error of the difference and the odds against occurrence by chance are extremely high.

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[1] Smithburn, K. C., *J. Exp. Med.*, 1936, **64**, 771.

* Sodium propyl-methyl-carbinyl allyl barbiturate, Lilly, supplied by Eli Lilly and Company, through Dr. G. F. Kempf, Indianapolis City Hospital.

The differences noted cannot be attributed to sexual differences in expectancy as pointed out by Berg[2] since the experiments were terminated quickly by the acuity of the disease; furthermore, the young animals of the second experiment, which might have been expected by Berg's criteria, to die the earlier, proved to be the more resistant. It should be emphasized also that the inoculating dose per animal was the same in mass. Calculating on the basis of mg of bacteria per kg of body weight, the young animals received 7.7 times the dose given to the old and 3.3 times the dose given to the middle-aged group. Since all other conditions of the experiment were the same in the 3 groups, the differences noted must have been due to variations in resistance with age.

Conclusion. Resistance to tuberculosis, as measured by survival-time of guinea pigs after intracerebral inoculation, varies with age. Young animals are the more resistant, and susceptibility increases progressively with age.

9943

Hypoglycemic Response of Patients Using Protamine Zinc Insulin to Induced Hyperglycemia.

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Some of the difficulties encountered in treating diabetes could be attributed to rapidly fluctuating blood sugar levels. The slow acceptance of protamine zinc insulin by the clinician was, in part, due to the occurrence of hypoglycemia episodes. They can be differentiated into two groups, the nocturnal, and the post-prandial. The former are obviously due to the unopposed prolonged action of Prozinsulin during the long fast from the evening meal to breakfast. An explanation for the latter type is given below.

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[2] Berg, W. N., *Am. Rev. Tuberc.*, 1938, **37**, 259.

Fifty-two diabetic patients using protamine zinc insulin exclusively for routine control were selected at random for the experiment. This consisted in the administration of 210 g to 420 g of orange juice, (25 to 50 g of glucose) 24 hours after the last insulin injection. Blood sugar estimations were made by the Somogyi (1936) modification of the Shaffer-Hartmann method in the fasting state, and at 0.5-hour intervals for 4 hours. The results shown in Table I with the controls, represent illustrative types of blood sugar variations seen in this series of experiments.

TABLE I.

				Mg glucose/100 cc blood							
Date	Patient	Gm orange juice	Fasting	½ hr	1 hr	1½ hr	2 hr	2½ hr	3 hr	3½ hr	4 hr
10-12	N.G.	420	192	224	254	259	216	171	160	154	149
9-2-37	E.S.	420	114	179	214	255	261	178	160	150	140
9-9	R.R.	420	219	260	287	294	266	178	155	119	109
10-2	A.M.	420	90	145	221	209	184	173	127	111	109
8-26	C.R.	420	86	165	210	194	140	132	99	80	77
10-6	J.Z.	420	205	273	299	279	244	209	190	197	129
9-10	N.R.	420	110	135	201	160	139	128	107	60	68
12-1	S.S.	420	334	380	339	326	324	311	303	263	257
9-3	S.B.	420	221	247	285	240	225	204	178	163	150
11-17	M.S.	420	134	197	252	227	173	141	105	105	104
10-13	S.F.	420	218	247	282	263	236	209	196	176	143
11-18	I.R.	420	193	203	247	244	228	212	184	179	173
8-13	S.S.	210	115	135	195	222	182	145	140	129	114
9-1	A.W.	420	97	111	137	125	104	73	71	65	62
12-20	E.G.	420	52	102	119	88	74	65	59	54	64
11-16	H.M.	250	107	125	151	182	165	155	136	119	94
8-27	J.L.	420	77	114	139	124	103	100	98	78	73
8-24	D.S.	210	80	125	200	153	128	120	110	80	62
11-13	D.D.	420	119	153	195	194	195	174	194	194	185
3-1	F.Y.	Control	133	133	158	156	164	150	148	153	154
3-1	L.P.	,,	125	127	133	138	122	124	126	128	130
11-1	M.B.	,,	190	160	155	151	150	177	180	200	215

It is evident that the peak rise in the first 0.5 to 1.0 hr and the subsequent fall to fasting, or below fasting levels by the 3.0 hr resembles the normal glucose tolerance curve. In fact, many of the curves cannot be distinguished both in absolute change and contour from non-diabetic ones. Another point to be noted is that the rapidity of the rise has a direct bearing on the rate of fall, independent of the original blood sugar level. That is, the sharpest peak rise in blood sugar level is followed by the most precipitous drop. During the test period, patients with such curves manifested hypoglycemic symptoms. It will be noted that in the control curves where no food was given to the patient, the blood sugar level remained essentially unchanged for the 4-hr period. It must be assumed that the amount of exogenous insulin present was the same both in the test period after orange juice, and in the control period. Apparently the rise in blood sugar level caused by the ingestion of glucose stimulated the glucose-disposing mechanisms. When the stimulus produces an exaggerated response the clinical symptoms of hypoglycemic shock appear.

It can be concluded that the too rapid rise in blood sugar concentration can be responsible for the post-prandial hypoglycemias. As a corollary, it may be stated that hypoglycemia can be avoided by dietary regulation directed toward slowing the availability of the carbohydrate. Evidence is being accumulated to show the practical clinical application of these conclusions.

9944

Blood Glucose and Lactic Acid in Relation to Milk Secretion.†

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The recent *in vitro* synthesis of lactose by Petersen and Shaw[1] from lactic acid and glucose and the demonstration by Graham[2] of the loss of lactic acid from the blood traversing the mammary gland indicates that lactose is synthesized in the gland from lactic acid and glucose of the blood.

Kaufman and Magne[3] found that in lactating animals the mammary venous blood contained less glucose than the jugular blood. Loss of glucose from the blood in passing through the lactating mammary gland has also been shown by Blackwood and Stirling,[4] Lintzel,[5] and Graham, Jones and Kay.[6] The latter workers reported that the amount of glucose taken out of the blood was proportional to the level of glucose in the arterial blood and to the milk yield per day.

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* The data in this paper are from a thesis presented by J. C. Shaw in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, Minnesota Agricultural Experiment Station.

[1] Petersen, W. E., and Shaw, J. C., *Science*, 1937, **86**, 398.

[2] Graham, W. R., Jr., *J. Biol. Chem.*, 1937, **122**, 1.

[3] Kaufman, M., and Magne, H., *Compt. Rend. Acad. Sci. Paris*, 1906, **143**, 779.

Several workers have demonstrated reduced blood and milk sugar by injection of insulin into cows, goats and sheep. Brown, Petersen and Gortner[7] reported that reducing the blood sugar to 17 mg % resulted in a drop in milk sugar to about one-fourth normal.

It has been much more difficult, however, to increase the lactose by increasing the blood sugar. Whitnah, Riddell and Hodgson[8] obtained only a slight increase in the lactose of milk when the blood sugar was raised to a very high level by pumping glucose solution into the stomachs of lactating cows. Likewise Brown, Petersen and Gortner[7] obtained only a very slight increase in lactose secretion by producing a hyperglycemic condition with intramammary duct injections of isotonic glucose. The sugar content of blood was raised to 300 mg % by Petersen and Boyd[9] by infusion of isotonic solutions of glucose into the external pudic artery without any increase in the lactose of the milk.

In a further study of the relation of blood glucose to milk secretion, a study was made of the relation of the blood glucose absorbed by the mammary gland to the following: time following completion of last milking, arterial blood sugar level, and level of milk production. A further study was made of the use of lactic acid by the mammary gland and the total blood carbohydrate needed for the elaboration of milk sugar.

Arterial and venous blood samples were taken simultaneously from the internal iliac artery or the external pudic artery and the subcutaneous abdominal mammary veins respectively. The method proposed by Graham, Kay and McIntosh[10] was followed in obtaining the arterial blood from the internal iliac. The method of Boyd and Petersen[11] was used in obtaining arterial samples from the same animal on several consecutive days.

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[4] Blackwood, J. H., and Stirling, J. D., *Biochem. J.*, 1932, **26**, 357.

[5] Lintzel, W., *Z. Zücht., B*, 1934, **29**, 219.

[6] Graham, W. R., Jr., Jones, T. S. G., and Kay, H. D., *Proc. Roy. Soc. London*, Series B, 1936, **120**, 330.

[7] Brown, W. R., Petersen, W. E., and Gortner, R. A., *J. Dairy Sci.*, 1936, **19**, 147.

[8] Whitnah, C. H., Riddell, W. H., and Hodgson, R. E., *J. Dairy Sci.*, 1933, **16**, 347.

[9] Petersen, W. E., and Boyd, W. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 537.

[10] Graham, W. R., Jr., Kay, H. D., and McIntosh, R. A., *Proc. Roy. Soc. London*, Series B, 1936, **120**, 319.

[11] Boyd, W. L., and Petersen, W. E., unpublished data, 1938.

TABLE I.
Glucose in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods.

Cow No.	Glucose level		Arterial Venous Difference, mg %	Remarks
	Arterial, mg %	Venous, mg %		
435	68.0	59.0	—9.0	2 hr after milking
432	63.6	53.4	—10.2	
446	63.6	58.4	—5.2	
577	76.0	71.0	—5.0	
434	68.8	66.4	—2.4	avg —6.36
435	65.0	56.0	—9.0	3 hr after milking
241	75.0	62.0	—13.0	
241	62.0	49.0	—13.0	
241	59.4	48.4	—11.0	
435	70.0	63.0	—7.0	
435	64.0	59.0	—5.0	
241	81.6	68.4	—13.2	
447	64.0	53.0	—11.0	
419	74.0	71.0	—3.0	
433	56.0	50.0	—6.0	avg —9.12
241	73.0	55.0	—18.0	4 hr after milking
241	71.0	60.6	—10.4	
241	74.4	63.0	—11.4	
241	59.6	51.0	—8.6	
578	52.0	45.6	—6.4	
26	47.8	39.4	—8.4	
423	51.2	47.8	—3.4	
423	78.8	63.0	—15.8	avg —10.30
435	58.0	44.0	—14.0	5 hr after milking
435	65.0	59.4	—5.6	
241	73.2	64.0	—9.2	
E-190	70.0	64.0	—6.0	
435	60.0	56.0	—4.0	
435	76.4	60.4	—16.0	
443	64.0	52.6	—11.4	
212	59.6	45.8	—13.8	
213	59.6	50.6	—9.0	avg —9.87
435	64.0	58.4	—6.0	9 hr after milking
E-190	78.0	71.0	—7.0	
235	76.0	65.6	—10.4	avg —7.8
435	61.0	56.0	—5.0	10 hr after milking
241	48.0	33.4	—14.6	
25	74.4	65.4	—9.0	
A-7	72.0	61.6	—10.4	
586	78.0	63.0	—15.0	avg —10.80
Avg	66.4	57.1	—9.3	

Lactic acid was determined by West's[12] modification of the von Fürth-Charnass technic.

The method used for blood sugar was essentially that recommended by Shaffer and Somogyi.[13] The blood filtrates were prepared by the Somogyi[14] method. Sodium citrate was used as an anticoagulant. Considerable precaution was exercised in obtaining the blood samples. In the event that there was apparent excitation of the animal the samples were discarded.

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[12] West, E. S., *J. Biol. Chem.*, 1931, **92**, 483.

The data for 40 arterio-venous glucose differences are presented in Table I. With the possible exception of the first 2 hours following milking there was no apparent relation between the arterio-venous glucose difference and the period of time following the completion of the last milking. The average arterio-venous blood sugar loss was 9.3 mg %.

A study was made of the relation of the arterio-venous glucose difference to the arterial blood sugar level and to the level of milk production. The results are given in Figs. 1 and 2. In both comparisons the A-V differences are so widely scattered as to hardly justify the plotting of a curve. The same situation prevails in the data presented in Table II in which several experiments were conducted on the same animals over a period of a few weeks. With the same individuals, neither the arterial blood sugar level nor the milk yield was related to the arterio-venous glucose difference.

The glucose and lactic acid concentration of arterial and mammary venous bloods are presented in Table III. There was a loss of lactic acid in 15 of the 17 experiments confirming the work by Graham[2] on lactating goats. The amount of glucose absorbed by

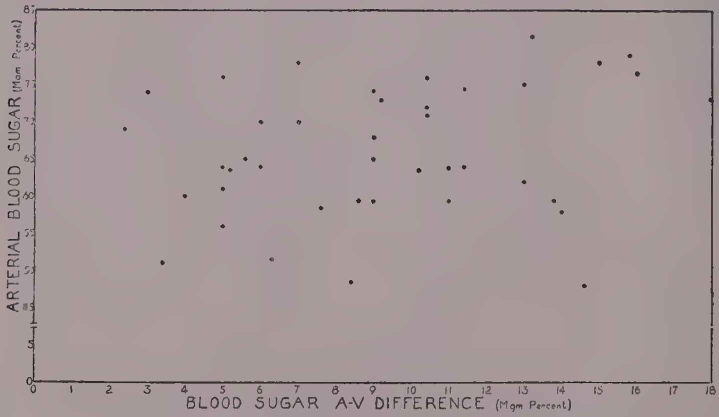

Fig. 1.
Relation of A-V difference to arterial blood sugar level.

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[13] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

[14] Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

TABLE II.
Arterio-Venous Blood Sugar Difference in Relation to Arterial Level of Blood Sugar and to Level of Milk Production.

Cow No. 241		Cow No. 435	
Arterial Blood Sugar Level mg %	A-V Blood Sugar Difference mg %	Milk Yield lb	A-V Blood Sugar Difference mg %
75.0	—13.0	19.6	—5.0
74.4	—11.4	16.6	—5.6
73.2	—9.2	15.6	—9.0
73.0	—18.0	15.1	—9.0
71.0	—10.4	10.1	—5.0
62.0	—13.0	9.6	—4.0
59.6	—8.6	8.8	—7.0
59.4	—11.0	8.6	—7.6
48.0	—14.6		

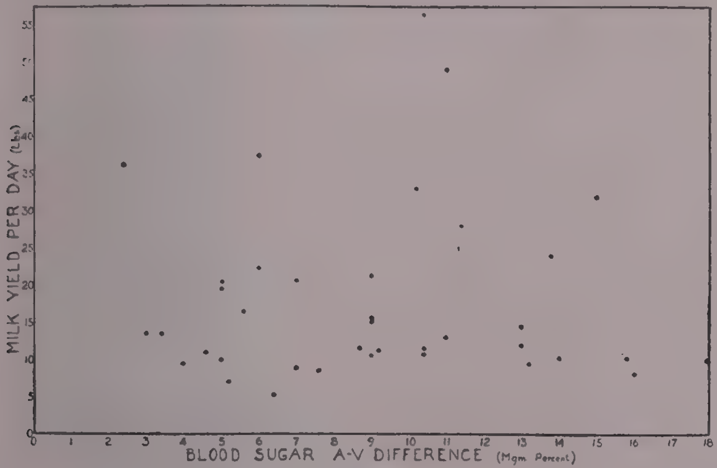

FIG. 2.
Relation of A-V difference to milk yield.

the lactating mammary was usually greater than that of the lactic acid. It will be noted that there is no apparent relationship between the glucose and lactic acid loss at any one time. The same thing will be observed in Graham's data. It is believed that this indicates a storage in the mammary gland of the precursors of lactose. During extreme excitement there is little or no loss in glucose and lactic acid and at times even a higher concentration in the venous blood of both substances.

The volume of blood flow as measured by the ratio of the calcium and fat removed by the gland to the percent of calcium and fat in

TABLE III.

Glucose and Lactic Acid in Arterial and Mammary Venous Blood of Cows.

Glucose			Lactic acid			
Arterial mg %	Venous mg %	Difference mg %	Arterial mg %	Venous mg %	Difference mg %	
63.6	53.4	—10.2	5.72	3.65	— 2.07	
64.0	53.0	—11.0	7.13	8.68	+ 1.55	
74.0	71.0	— 3.0	5.94	3.47	— 2.47	
76.0	71.0	— 5.0	12.97	6.21	— 6.76	
68.8	66.4	— 2.4	7.67	6.58	— 1.09	
56.0	50.0	— 6.0	7.58	3.29	— 4.29	
74.4	63.0	—11.4	9.04	5.30	— 3.74	
74.4	65.4	— 9.0	16.63	6.21	—10.42	
72.0	61.6	—10.4	8.40	3.38	— 5.02	
78.0	63.0	—15.0	3.93	5.48	+ 1.55	
59.6	51.0	— 8.6	19.46	11.69	— 7.77	
52.0	45.6	— 6.4	9.04	5.62	— 3.42	
47.8	39.4	— 8.4	8.45	4.25	— 4.20	
51.2	47.8	— 3.4	6.76	3.79	— 2.97	
78.8	63.0	—15.8	8.59	4.93	— 3.66	
59.6	45.8	—13.8	7.93	5.39	— 2.54	
59.6	50.6	— 9.0	9.04	4.48	— 4.56	
71.2	72.0	+ 0.8	7.64	8.40	+ 0.76	cow* excited
60.6	63.0	+ 2.4	4.69	5.33	+ 0.64	dry cow
		Avg of lactating cows				
65.3	56.5	— 8.8	9.08	5.44	— 3.64	

*Not included in average.

the milk has been shown by Shaw and Petersen[15] to be in the range of 391 :1 and 344:1 respectively. The average arterio-venous glucose loss of 9.3 mg % as given in Table I when compared to the average lactose in the milk indicates a ratio of 550 volumes of blood for each unit volume of milk produced. It is, therefore, apparent that the glucose is not removed by the gland in sufficient quantities to account for all of the lactose in the milk. The volume flow of blood per unit volume of milk calculated from the combined average glucose and lactic acid differences as given in Table III and the average lactose percent in milk is about 390 which is in very good agreement with the values calculated from calcium and fat as cited above. Thus the combined glucose and lactic acid loss to the mammary gland appears to be sufficient to account for all of the milk sugar.

This confirms in part the observation by Graham[2] that the blood glucose is not the sole precursor of lactose and that lactic acid is also one of the precursors of lactose. Graham conducted a carbohydrate balance experiment on the mammary gland of a goat. While very good agreement was obtained in the two experiments reported, many more data are essential for conclusive proof.

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[15] Shaw, J. C., and Petersen, W. E., *Proc. Am. Physiol. Soc.*, 50th Annual Meeting, Baltimore, Maryland, 1938, p. 183.

Conclusions. 1. There was no apparent relation between arterio-venous blood sugar differences and the following: period of time following milking, arterial blood sugar level and level of milk production. 2. Both glucose and lactic acid are absorbed by the lactating mammary gland in considerable quantities. While the glucose arterio-venous loss is not sufficient to account for all of the milk sugar, the combined loss of the two substances to the lactating mammary gland can account for all of the milk lactose.

9945 P

Stimulation of Reproductive Tract of the Infantile Female Mouse by Anuran Anterior Pituitary Substance.*

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Attempts to stimulate the reproductive tract of the infantile female mouse by small doses of anuran anterior pituitary substance have been consistently negative.[1] Even with larger amounts, *e.g.*, 16 to 96 fresh anterior lobes of *Rana pipiens,* averaging 16.2 to 104.0 mg per mouse, the ovaries, uteri and vaginae showed no differences compared with litter mate controls although thyroid and adrenal glands were stimulated to a degree that was significant statistically.[2]

In the present experiments, however, a positive reaction has been secured by approximately doubling the highest dosage of fresh pars anterior previously employed. Infantile female mice, 17 to 21 days old, were used as hosts and *Rana pipiens* as donors of the anterior pituitary substance. The frog anterior lobes were removed and placed in a bottle containing 0.3 cc of 0.9% NaCl packed in ice. As soon as a certain amount of pars anterior substance, judged by number or weight of glands, was secured, it was macerated and then injected into the hind leg of the mouse. Any tissue remaining in the bottle was injected into the other leg in an additional 0.3 cc of salt solution. Equivalent doses of frog brain and smaller amounts

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* Aided by a grant from the Rockefeller Foundation.

[1] Lipschütz, A., and Paez, R., *C. R. Soc. Biol.*, 1928, **99**, 693; Martins, Th., *C. R. Soc. Biol.*, 1929, **101**, 957; Zondek, B., *Arch. Gynäk.*, 1931, **144**, 133; *Hormone des Ovariums und des Hypophysenvorderlappens*, 2nd edit., J. Springer, 1935; Magistris, H., *Pflüger's Arch.*, 1932, **230**, 835.

[2] Adams, A. E., and Tukey, G., *Anat. Rec.*, 1938, **71**, in press.

of mouse pars anterior or brain were administered similarly to litter mates. Treatment was given daily for 3 or 4 days and the animals were autopsied on the fourth or fifth day following the first treatment.

Table I gives the average data of tests made on 5 litters of mice.

TABLE I.
Averaged Weights of Reproductive Tracts of Infantile Female Mice from 5 Litters Given Fresh Frog Anterior Pituitary Substance, Fresh Mouse Anterior Lobes, Fresh Frog or Mouse Brain or Left Untreated.

			Wt at autopsy, mg			
No. of animals	Body wt at autopsy, gm	Treatment	Ovaries	Tubes, uterus, vagina	Tubes, uterus	Vagina
6	8.6	223.3 mg frog A.P. per mouse (211.5 ant. lobes)	4.88	49.87	30.99	18.88
5	10.0	5.41 mg mouse A.P. per mouse (4.8 ant. lobes)	3.89	38.47	18.18	20.29
12	9.6	233.0 mg frog brain per mouse (5 mice) 4.14 mg mouse brain per mouse (2 mice) Untreated (5 mice)	2.50	15.16	7.04	8.12

Four of the 6 individuals receiving frog pars anterior showed very high weight increases in all parts of the reproductive tract and the histological picture of ovaries, uteri and vaginae of these revealed, respectively, increase in numbers of Graafian follicles and follicles with antra, marked thickening of uterine walls and vaginal epithelium characteristic of proestrus or estrus with an open vagina in the estrous stage. In the other 2, the response was less pronounced. The thyroids and adrenals of the 6 animals also were stimulated. Tests were made with very large doses of acetone-dried pars anterior of frogs and a positive but less marked reaction was observed in the reproductive tracts of 7 of 9 individuals so treated, but in all thyroids and adrenals responded. The reproductive tracts of all of the mice given mouse pars anterior reacted positively and the vaginal picture was that of estrus or metestrus. No stimulation occurred in mice given frog brain, mouse brain or left untreated.

Summary. Stimulation of the reproductive tracts of infantile female mice has been brought about by the administration of large amounts of fresh anterior pituitary from *Rana pipiens*. It is believed that this is the first time precocious sexual maturity has been caused in the mammal by anuran pars anterior.

9946

Blood Lactic Acid After Exercise with Particular Reference to Polycythemia Rubra Vera.

Phillip Hallock. (Introduced by Maurice B. Visscher.)

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Under normal resting states the concentration of lactic acid in venous blood of normal human beings varies from 10 to 20 mg per 100 cc of blood where the Friedman Contonio and Shaffer method is used. (Peters and Van Slyke).[1] When normal individuals are submitted to mild exercise* a slight rise of the lactic acid concentration above the resting level occurs. (Table I.) The average concentration of the lactic acid values was 13.9 mg per 100 cc at rest while after mild exercise it rose to 16.3 mg per 100 cc of blood, an average increase of slightly more than 2 mg per 100 cc. In no instance did the lactic acid concentration exceed 21 mg %. In these 11 cases the hemoglobin concentration and red blood counts were in the normal range.

In Table II we have recorded the resting and post exercise values of the blood lactic acid in 5 normal individuals with high hemoglobin

TABLE I.
Lactic Acid Content of Venous Blood During Rest and After Exercise in Normal Subjects.

Case	Age	Sex	Amount of work performed kg	Lactic acid (mg per 100 cc) before	after
1	22	Female	1,260	12.8	15.0
2	23	Male	1,440	10.3	12.9
3	11	Female	1,050	13.6	16.6
4	19	”	1,287	11.4	13.7
5	13	Male	1,350	15.0	17.0
6	24	”	1,920	15.8	16.4
7	13	Female	1,092	12.6	16.8
8	59	Male	2,000	15.3	20.5
9	10	Female	350	12.0	17.2
10	24	Male	1,700	15.4	15.8
11	35	”	1,500	17.7	17.8
			Avg	13.8	16.3

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[1] Peters, J. P. and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. I, Williams and Wilkins Co., 1932.

* The exercise test consisted of having the subject step on and off a stool exactly one foot high. The duration of the exercise varied from 4 to 6 minutes depending upon the number of steps the subject was obliged to take in order to complete the required amount of work.

TABLE II.
Lactic Acid Levels of Venous Blood Before and After Exercise in Normal Individuals with High Red Counts and Hemoglobin Contents.

Case	Age	Sex	Work performed, kg	Lactic acid, mg %		Hgb.	RBC million
				before	after		
1	23 (JF)	Male	1,526	19.06	21.44	111	5¾
2	27 (CG)	”	1,560	12.01	12.35	108	6
3	21 (DS)	”	1,440	16.25	19.34	99	5
4	25 (RK)	”	1,530	12.20	12.83	106	5
5	30 (AM)	”	1,600	16.32	20.63	108	5
6	26 (WS)	” (diabetic)	1,520	19.58	22.26	105	5½
			Avg	15.0	17.3		

and red counts including one diabetic with a high hemoglobin and red blood count. Excluding the latter the average resting value as well as the average values following exercise are higher than those in the normal range. As in normals a slight but definite rise in the lactic acid concentration takes place, going from an average resting value of 15 mg % to 17.3 mg %, an average increase of slightly over 2 mg %. From this it is evident that normal individuals with high hemoglobin contents and high red blood counts show essentially the same lactic acid response to exercise as normal individuals.

Extreme grades of secondary polycythemia may often be found in association with morbus caeruleus type of congenital heart disease, where the direction of the shunt is venous-arterial. Table III shows the lactic acid response to exercise in those cases of congenital heart disease with cyanosis, where in some cases polycythemia was one of the outstanding features.

It will be noted from the Table III, that in some instances the lactic acid concentration doubles itself after exercise. However, a consistent rise of the blood lactic acid is found in all cases. The polycythemia of congenital heart disease appears to be a compensatory process arising from the stimulation of erythropoietic tissue of bone marrow, in an attempt to reduce the oxygen deficit by providing more hemoglobin and red cells.

The polycythemia found in extreme cases of emphysema is likewise regarded as a compensatory process to combat anoxemia. In Table IV we have recorded the lactic acid response to exercise in emphysema. Cases 1 and 2 showed a slight polycythemia. Here again we see a rise in lactic acid, but not so great as seen in morbus caeruleus.

We next investigated the lactic acid response to exercise in cases of polycythemia rubra vera. We have studied 9 cases (Table V). All of

TABLE III.

Lactic Acid Levels of Venous Blood Before and After Standard Exercise in Those Cases of Congenital Heart Associated with Venous-Arterial Shunts.

Case	Age	Sex	Diagnosis	Work performed, kg	Lactic acid, mg %		Hgb. %	RBC m.
					before	after		
11	63	Male	Intervent. septal defect	1200	18.44	43.25	121	7½
12	19	”	Tetralogy of Fallot	540	17.98	40.94	106	11
13	17	Female	” ” ”	1350	16.03	28.44	132	7½
14	23	”	Patent ductus	875	13.20	28.68	90	5
14	23	”	” ”	984	13.30	27.54		
15	21	”	Intervent. septal defect probably associated with patent ductus	1475	20.88	47.10	82	5
15	21	”	Intervent. septal defect probably associated with patent ductus	1720	18.48	29.56		
16	25	Male	Intervent. septal defect with patent ductus	1512	20.78	22.01	130	7
16	25	”	Intervent. septal defect with patent ductus	1800	18.85	22.57		
				Avg	17.5	32.2		

TABLE IV

Lactic Acid Levels of Venous Blood Before and After Exercise in Emphysema.

Case	Age	Sex	Amount of work kg	Lactic acid, mg %		Hgb.	RBC million
				before	after		
1	60 (MM)	Male	1409	17.9	20.1	104	5
2	51 (H)	”	819	20.5	24.7	105	5½
3	41 (MLM)	”	1320	21.2	22	86	4½
			Avg	19.8	22.2		

these patients exhibited the classic clinical picture of polycythemia rubra vera. The diagnoses were verified by blood studies, mainly hemoglobin concentration, red blood cell count, hematocrit readings and blood volume determinations. It will be noted that a most unusual lactic acid response develops following mild exertion. Instead of the blood lactate increasing as it does in normals, and in other types of secondary polycythemia, we find in these cases on exercise an actual decrease in the lactic acid concentration of the blood. The average resting blood lactate value is 17.8 mg %, while following exercise it declines to 15.1 mg %, an average decrease of 3 mg %. This decrease was found to be consistent in all cases. That the decrease in the concentration of the blood lactate is not dependent on the height of the hemoglobin and red blood cell content of the blood, is indicated in case 8. This patient was first admitted to the University Hospital in 1930, and a diagnosis of polycythemia rubra

TABLE V.
Lactic Acid Levels of Venous Blood Before and After Exercise in Polycythemia Rubra Vera.

Case	Age	Sex	Work performed, kg	Lactic acid, mg %		Hgb. %	RBC m.	B.V.	H crt, %
				before	after				
1	45 (P)	M	1,440	19.2	14.1	112	8		
2	55 (ER)	M	1,526	16.4	14.1	110	6		
3	49 (JS)	M	1,700	18.6	15.6	114	7¼	7.0 L.	65
3 (a)			3,000	16.4	15.3	99	5¼		
			(following phenylhydrazine therapy)						
4	51 (ME)	Fe	1,300	20.1	17.4	100	6¾	5.2 L.	66
5	30 (MC)	M	1.585	19.0	13.3	122	6		
6	49 (JM)	M	1,500	15.4	12.9	129	6¼	8.1 L.	64
7	27 (EH)	M	1,550	18.2	16.7	101	5	6.2 L.	55
7 (a)			3,000	16.2	15.6	99	6		
8	50 (AP)	Fe	1,400	19.2	16.3	78	4¾	4.3 L.	41
			(myelogenous leukemia)						
9	24 (ML)	M	1,560	22.1	17.5	126	7	6.1 L.	65
9 (a)			2,000	13.9	12.7	90	4¾		50
			(spontaneous remission)						
			Avg	17.8	15.1				

vera was made. The hemoglobin at that time was 120%, and the red blood count 7.5 millions. She was referred to the University Hospital for further study in March, 1938, because of anemia and progressive enlargement of the spleen. On this second admission, she was found to have myelogenous leukemia, a complication which not infrequently follows from polycythemia rubra vera.[2] Her hemoglobin now was 78% and red blood count 4.5 millions.

When this patient was submitted to the lactic acid test, a typical drop in the blood lactate occurred. This case is extremely instructive, inasmuch as the blood lactic acid values indicate that the polycythemic state is not necessary to produce the drop in the blood lactate. This observation was further corroborated in cases of polycythemia rubra vera whose hemoglobin and red blood count levels were lowered to within the normal range by the therapeutic administration of phenylhydrazine. Case 3 is such an example.

The lactic acid response was obtained before treatment, when the hemoglobin was 114% and the red blood count 7.5 millions. The patient was then treated with the drug and the hemoglobin and red blood count brought down to 99% and 5.5 million cells respectively [3(a)]. The same response was again noted, namely a lowering of the blood lactate level after exercise. Case 9 also illustrates the same point [see 9(a)]. Furthermore the drop in the lactic acid level was obtained when patients with true polycythemia were submitted to more strenuous exercise. Thus, as indicated in Table V, when patients 3, 7 and 9 were submitted to increased work 3(a), 7(a), and 9(a), the same response was still obtained. From these observations we can conclude that the hemoglobin and red blood count level is not the factor which is responsible for the decrease in the blood lactate level in true polycythemia, inasmuch as the response is still obtained at lower ranges.

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[2] Minot, G. R., and Buchman, T. E., *Am. J. Med. Sciences*, October, 1923, **166**, 469.

It is believed that these observations indicate that at rest, the blood flow, particularly through the muscles and perhaps certain glands producing lactic acid, is diminished below normal levels, and that anaerobic metabolic processes lead to the accumulation of abnormal amounts of lactic acid in the blood. With mild exercise the blood flow through muscle is greatly increased and the oxygen supply raised enough to prevent the lactate production. This view is supported by the additional observation that a polycythemic patient responded to a hot bath of 15 minutes' duration by a decrease in blood lactic acid. The fact that the same response occurs in the polycythemia rubra vera patient after reduction of the red cell count to normal, as when the count was elevated, indicates that the disturbance in lactate metabolism is dependent upon some primary factor such as a vasoconstriction, to which the polycythemia is itself secondary. These results seem to indicate, therefore, that tissue anoxia may be an important link in the chain of pathological processes involved in polycythemia vera, and that the primary disturbance may be a persistent vasoconstriction in certain tissues.

9947 P

Artificially Produced Anoxia as a Means of Demonstrating Abnormal Respiratory Function.

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A considerable degree of pulmonary collapse can be maintained in man with very little alteration of respiratory function other than an abnormal amount of hyperventilation on exertion. Latent physiological abnormalities usually become manifest clinically only under stress. The response to exercise is too dependent upon physical fitness to be a reliable guide to the capacity of an individual to compensate for a reduction of lung volume. Since oxygen lack constitutes the greatest danger to patients who have disorders of respiration, the oxygen saturation of the arterial blood while breathing a mixture of gases containing less oxygen than the atmosphere would seem to furnish a simple and quantitative test of one phase of respiratory efficiency while the subject remains at rest.

The procedure consists in the analysis of the alveolar air and arterial blood while the subject breathes room air and again while he breathes from a Douglas bag containing about 16% oxygen in nitrogen. By this means it is possible to ascertain the degree of arterial saturation produced by the observed alveolar oxygen tension. Alveolar air was withdrawn in 1 or 2 cc portions from the axial stream in the center of the mouthpiece of a set of respiration valves at the end of successive expirations until 40 cc or more were obtained. This is essentially a modification of the method of Krogh and Lindhard[1] which was shown by Debenham and Poulton[2] to give results which agree with Haldane-Priestley samples. Air analyses were done in the Haldane-Henderson apparatus.

Arterial puncture is often painful and frequently causes patients to hold their breath or hyperventilate, and full reliance cannot always be placed upon the analysis of blood obtained by this means.[3] Since 2 samples of blood are taken within a few minutes of each other in this procedure, the method of Goldschmidt and Light[3] was used. In this method the forearm is immersed in water at 45-47°C for 10 minutes and blood is drawn with gentle suction from one of the veins on the dorsum of the hand. Since the tissues from which it comes are relatively low in metabolism and the vessels are sufficiently superficial to allow the heat to be effective in dilating most, if not all of them, the blood obtained is, as far as gas content is concerned, indistinguishable from that taken directly from an artery. Repeated sampling at one time is also possible. Blood gas analyses were done in the manometric apparatus of Van Slyke and Neill.

The average values for the observed saturation, the calculated saturation, and the difference between the two while the subjects breathed room air and 16% oxygen in nitrogen respectively, are given in Table I. For convenience in analysis, the 52 patients with

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[1] Krogh, A., and Lindhard, J., *J. Physiol.*, 1913-14, **47**, 431.

[2] Debenham, L. S., and Poulton, E. P., *Quart. J. Med.*, 1918-19, **12**, 38.

[3] Goldschmidt, S., and Light, A. B., *J. Biol. Chem.*, 1925, **64**, 53.

TABLE I.

Difference Between Observed and Calculated Arterial O_2 Saturation at Observed Alveolar O_2 Tension with Subject Breathing Room Air and 16% O_2 Respectively.

		Room Air				16% Oxygen				
			Arterial O_2 saturation, %				Arterial O_2 saturation, %			
Group	No. of cases	Alv. O_2 tension mm Hg	Observed	Calculated	Dif. D_1	Alv. O_2 tension, mm Hg	Observed	Calculated	Dif. D_2	D_2-D_1
Normals	10	103.6 (96.1-111.9)	98.1 (96.3-99.3)	98.8	0.7	69.3 (61.4-79.2)	93.1 (89.4-96.4)	93.8	0.7	0.0
I	18	105.2 (98.2-117.0)	97.7 (95.8-99.3)	98.9	1.2	74.8 (68.4-83.8)	91.6 (90.0-93.0)	95.1	3.5	2.3
II	18	103.7 (89.1-112.1)	97.0 (94.3-98.8)	98.8	1.8	73.0 (64.2-83.6)	87.7 (85.2-89.6)	94.6	6.9	5.1
III	16	102.0 (89.6-110.8)	93.9 (87.0-98.0)	98.7	4.8	71.3 (64.6-78.4)	80.8 (56.7-84.9)	94.3	13.5	8.7

The figures in parentheses represent the extreme range of variation.

pulmonary disease (of whom 42 had various forms of unilateral and bilateral pulmonary collapse, 6 had tuberculosis without collapse and 4 had silico-tuberculosis without collapse) are divided into 3 groups on the basis of their ability to saturate their arterial blood while breathing the low-oxygen mixture: Group I (18 patients), above 90%: Group II (18 patients), between 85% and 90%; and Group III (16 patients), below 85%. The factors D_1 and D_2 represent in each case the difference between the experimentally determined arterial saturation and the saturation which should exist at its respective alveolar oxygen tension (calculated from an oxygen dissociation curve). The fact that the average values for the normal group (15 determinations on 10 subjects) fall within 0.7% of the curve establishes the validity of these methods of sampling and analysis.

It will be noted that there is a progressive increase in the values of both D_1 and D_2, indicating a failure on the part of the abnormal subjects to attain a degree of saturation commensurate with the alveolar oxygen tension. It will also be noted that this failure to maintain a normal oxygen tension in the arterial blood is strikingly magnified by breathing 16% oxygen in nitrogen. It would seem, therefore, that functional abnormalities which are not apparent under normal conditions can be brought to light by a respiratory strain without the complicating factor of lactic acid production and oxygen debt resulting from exercise. The discrepancy between actual arterial saturation and the saturation which should obtain at the existing alveolar oxygen tension suggests that the diffusion of oxygen through the pulmonary epithelium is retarded in the presence of disease and, to a much lesser extent, collapse of one or both lungs. Whether this is due to a change in the membranes themselves or to an alteration in the ratio between circulation and ventilation, either local or general, is now under investigation.

9948 P

Variations in Pathways by Which Equine Encephalomyelitic Viruses Invade the CNS of Mice and Guinea-pigs.

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Eastern equine encephalomyelitic (E.E.E.) virus, injected into the leg muscles of 15- to 21-day-old mice, invades the central nervous system (CNS) along the local peripheral nerves in only about 5% of the animals, while in most of the others it appears to be eliminated from the blood onto the olfactory mucosa, invading the CNS by the olfactory pathway, and when that pathway is blocked by preliminary chemical treatment of the nasal mucosa most, but not all, of the animals escape CNS involvement.[1] Further study by the method of partial serial sections of the entire CNS[2] revealed that when mice, given the virus intranasally, succumb in spite of the chemical treatment, the lesions are still distributed along the olfactory pathways and connections, showing that the treatment did not provide a complete barrier. When, however, mice receiving the virus *intramuscularly* succumb in spite of chemical blockade of the olfactory pathway, the localization of lesions indicates that the CNS was invaded by other pathways, the following types having been encountered: (a) lesions limited to the spinal cord and medulla, indicative of spread *via* the nerves supplying the inoculated muscle; (b) lesions involving chiefly the cochlear ganglion of one side and the inferior colliculus and medial geniculate body of the opposite side (*i.e.*, a unilateral involvement of the auditory pathway and its central connections); and (c) lesions limited to a focus in the vicinity of the medullary nucleus of the 7th nerve. Hence, these and other pathways may occasionally be utilized by virus injected into the leg muscles (and also intraäbdominally) of normal mice as well as of those with chemical blockade of the olfactory pathway. In one normal young mouse injected intraäbdominally with E.E.E. virus both the auditory and vestibular pathways appeared to be involved. It is remarkable, however, that in these mice the localization of lesions practically always corresponded to some definite pathway, suggesting that the virus was set free in one focus from which it spread along the nervous pathways connected with it (excepting the olfactory pathway, there is as yet no evidence to indicate whether that focus is within or outside the CNS) rather than liberated indiscriminately throughout the CNS.

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[1] Sabin, A. B., and Olitsky, P. K., *Am. J. Path.*, 1937, **13**, 615.

[2] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **67**, 201; Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 270.

A study of the localization of lesions in normal guinea pigs which were given mouse-passage E.E.E. virus into the leg muscles revealed, however, this diffuse distribution of lesions yielding a picture which in its totality has not hitherto been encountered in mice. The most striking difference is in the involvement of the neopallial cortex which in the mouse only rarely shows lesions and then only as limited foci bearing some relationship to an affected pathway, while in the guinea pig there are numerous lesions which are diffusely scattered, bearing a relationship only to the blood vessels which themselves show evidence of injury. These perivascular lesions frequently consist of almost complete neuronal necrosis with an abundant infiltration of polymorphonuclear leucocytes, while in many instances large numbers of polymorphonuclear leucocytes are present around the vessels without any evidence of neuronal necrosis. This diffuse, perivascular, neuronal necrosis in the guinea pig's neopallial cortex corresponds so closely to the type of lesion produced by toxoplasma[3] growing through the vessels in the brains of mice, that it strongly suggests that in the guinea pig E.E.E. virus also grows through the vessels and involves the nerve cells around them, a possibility already considered by Hurst[4] on experimental grounds. In addition there is usually sufficient neuronal necrosis along definite parts of the central olfactory connections to suggest that progression also occurred along this pathway. In only one instance was there flaccid paralysis of the posterior extremities following inoculation of the virus into the leg muscles (the only instance with extensive involvement of the spinal cord) but even in this animal there were concomitant signs of encephalitis with widely scattered lesions in the neopallial cortex.

With *Western* mouse-passage equine encephalomyelitis virus (W.E.E.) a somewhat different course was observed following intramuscular injection in both mice and guinea pigs. Among 15-day-old mice, 80 to 90% of the animals exhibit only signs of encephalitis (pathology indicative of invasion along olfactory or other pathways without evidence of either diffuse hemato-encephalic spread or progression *via* the local nerves), and only 10 to 20%, signs of flaccid paralysis (with pathological evidence of spread only by way of the local peripheral nerves). Among 21-day-old mice, however, 70 to 80% develop flaccid paralysis, 10 to 20% exhibit only signs of encephalitis, and the few remaining ones show no evidence of CNS disease. About half the number of 7- to 10-day-old guinea pigs injected into the leg muscles with the mouse-passage strain of W.E.E. exhibit only signs of encephalitis; an occasional young animal shows no nervous signs at all but most of the others reveal flaccid paralysis of the posterior extremities (first on the inoculated side) with pathological evidence of spread only along the local peripheral nerves and none of the diffuse vascular and perivascular lesions in the neopallial cortex. Since the capacity to produce such a high incidence of flaccid paralysis following injection into the leg muscles has not been noted previously in work on W.E.E., it is not improbable that it may be related to the number of mouse passages our strain of virus has undergone.

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[3] Sabin, A. B., and Olitsky, P. K., *Science*, 1937, **85**, 336.

[4] Hurst, E. W., *J. Path. and Bact.*, 1936, **42**, 271.

9949 P

Age of Host and Capacity of Equine Encephalomyelitic Viruses to Invade the CNS.

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Studies on the nature of various types of natural resistance to viruses affecting the central nervous system (CNS) have already disclosed that as mice and guinea pigs grow older there appear certain localized barriers in the peripheral and central nervous systems which, because of the nature of neural progression of peripherally injected vesicular-stomatitis virus, are capable of preventing it from invading the CNS or from spreading beyond a certain point, depending upon the route of inoculation.[1] Still another type of change that occurs with age was revealed in the course of investigations on the manner in which the viruses of equine encephalomyelitis (E.E.) invade the CNS after inoculation into the muscles of an extremity. This change which appears to involve the blood vessels, which the E.E. viruses must traverse or grow through in order to invade the CNS,[2, 3, 4] is the subject of the present report.

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[1] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1937, **66**, 15, 35; 1938, **67**, 201, 229.

[2] Sabin, A. B., and Olitsky, P. K., *Am. J. Path.*, 1937, **13**, 615.

[3] Sabin, A. B., and Olitsky, P. K., Proc. Soc. Exp. Biol. and Med., this issue.

[4] Hurst, E. W., *J. Path. and Bact.*, 1936, **42**, 271.

With the Eastern strain of the mouse-passage virus 15-day-old mice are 100% susceptible (intramuscular or intraäbdominal inoculations) while at one month of age 40 to 50% are already resistant and beyond the age of 3 months this resistance rises to 95%. Studies on the nature of this resistance revealed that while the virus invades (and persists or multiplies in) the circulating blood as in young mice, it apparently cannot get across the vessels, which for this infective agent seems to be in most instances a necessary preliminary step to involvement of the nervous system. It has already been shown[5] that young and old mice are practically equally susceptible to intracerebral and intranasal administration of the virus further suggesting that some change in the blood vessels is probably responsible for the resistance of the older animals. It is of interest to note that, as with vesicular-stomatitis virus,[1] the majority of old mice which are resistant to intramuscular inoculation succumb when the E.E.E. virus is injected directly into the sciatic nerve. In guinea pigs inoculated intramuscularly with 10^8 or 10^9 mouse-cerebral lethal doses (M.C.L.D.) no difference was observed with age, while with 10^7 or fewer M.C.L.D., old guinea pigs (800-1000 g) resisted in simultaneous tests in which 7- to 10-day-old animals (60-100 g) succumbed; one of the old guinea pigs injected with the smaller dose developed flaccid paralysis of the posterior extremities and exhibited lesions indicative of invasion along the nerves supplying the inoculated muscle, but not the diffuse and widely scattered vascular and perivascular changes ordinarily encountered in this species.[3]

With the Western strain of the mouse-passage virus (W.E.E.) the first evidence that age has produced some change in the animal is found in the fact already noted[3] that the majority (80 to 90%) of 15-day-old mice develop signs of encephalitis and only a few animals signs of flaccid paralysis after inoculation of the virus into one of the posterior extremities, while at 21 days of age the ratio is reversed, the majority developing flaccid paralysis and some (6 of 30) resisting altogether. At 1 month of age* and beyond, 90% or more of mice are resistant to intramuscular inoculation, (of 36 mice older than 6 months only one succumbed) although direct injection into the sciatic nerve supplying these muscles again results in the paralytic disease in a large proportion of the animals. There are thus at least 3 phases during the maturation of the mouse at which the neuroinvasiveness of W.E.E. virus is differently affected: first, at the very early age of 15 days when certain vessels still permit the virus to traverse and spread in the CNS by some definite pathway;[3] second, at the age of 21 days when the virus can no longer do this in the majority of mice but now progresses along the nerves supplying the inoculated muscle; and third, between the 21st and 30th days of life when the appearance of some change in the muscle or specialized nerve endings in the great majority of mice (it is important to remember the few exceptions) now prevents invasion of the CNS altogether. Intramuscular injections of as much as 10^8 M.C.L.D. of the mouse-passage W.E.E. virus finds practically all old guinea pigs (800-1000 g) resistant with only an occasional one exhibiting flaccid paralysis.

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[5] Olitsky, P. K., Sabin, A. B., and Cox, H. R., *J. Exp. Med.*, 1936, **64**, 723.

* Resistance to intramuscular injection of vesicular stomatitis virus also appears at this age and is just as marked.

Conclusions. Studies of the conditions which permit or prevent the viruses of vesicular stomatitis and equine encephalomyelitis to invade the CNS of mice and guinea pigs have thus far disclosed that depending upon the age or species of the host there may be changes or variations in localized sites, *e.g.*, in terminal, specialized nerve endings, in special areas of the CNS, blood vessels, etc., which can prevent the virus from invading or progressing in the CNS—changes in different tissues functioning as barriers for different viruses depending upon the mode of CNS invasion utilized by the virus.

9950 P

Inhibition of Estrogenic Effects on the Skeleton by Testosterone Injections.*

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Hypercalcification of the skeleton involving a partial or complete obliteration of the marrow cavities occurs in immature rats and chickens[1] and in mice[2] following extended treatment with large amounts of estrogens. Simultaneously with this, the pubic and ischial bones are reabsorbed at the symphysis and the former carti-

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* This investigation has been supported by grants from the Anna Fuller Fund, Fluid Research Fund of Yale University School of Medicine, and the Committee for Research in Problems of Sex of the National Research Council.

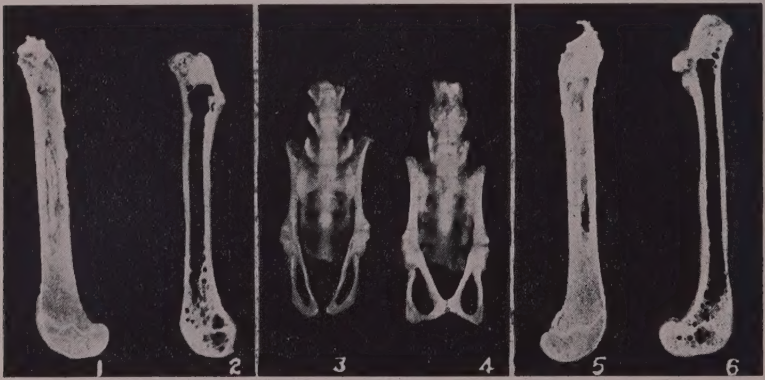
[1] Zondek, B., *Folia Clin. Orient.*, 1937, **1**, 1.

laginous union is replaced by an interpubic ligament.[2, 3] Similar pelvic changes occur during normal pregnancy.

Untreated old virgin females show only a slight loosening of the pelvis. However, ovarian grafts produce slight pelvic changes in males but are more effective in castrated than intact animals. Also in over 100 mice treated with estrogens the bone changes occurred more slowly in the intact males than in the females, indicating that the male hormone may exert an inhibiting effect. Therefore, testosterone propionate was injected along with the estrogen.

Six mice from 2 inbred strains were given weekly injections of 500 or 1000 i.u. of estradiol benzoate† and of 1.25 or 2.5 mg of testosterone propionate (Table I).

The femoral marrow cavities of mice receiving testosterone and estrogen were similar to those of normal untreated mice of comparable ages or showed only a slight increase in endosteal cancellous bone. (Figs. 2 and 6). Mice of the same strains receiving similar amounts of estrogen but no testosterone for comparable periods showed an extensive hyper-calcification of the femurs (Figs. 1 and 5), compact and cancellous bone largely replacing the marrow cavities.



Figs. 1 and 5. Femurs of a female (1) and a male (5). ($7C_3H$ and $6C_3H$ respectively. See table. Alizarine was injected during the last 24 days.

Figs. 2 and 6. Femurs of mice $91C_3H$ and 61N respectively (see table). These bones were similar to those of normal animals.

Figs. 3 and 4. Pelves of (3) 56A and (4) of $24C_3H$ (see table).

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[2] Gardner, W. U., and Pfeiffer, C. A., Proc. Soc. Exp. Biol. and Med., 1938, **37**, 678.

[3] Gardner, W. U., *Am. J. Anat.*, 1936, **59**, 459.

† The estrogen (Progynon B) and testosterone propionate (Oreton) were generously supplied by Drs. E. Schwenk and M. Gilbert of the Schering Corporation.

TABLE I.

	Sex	Days treated	Estrogen weekly, i.u.	Total estrogen, i.u.	Testosterone propionate, weekly, mg	Total testosterone propionate, mg	Pelvis	Femur
$24C_3H$	F	232	500	16,000	2.5	80	Firm (Fig. 4)	Normal
$88C_3H$	M	128	1000	18,000	1.25	21.25	,,	,,
$89C_3H$	M	127	1000	18,000	1.25	22.25	,,	,,
$91C_3H$	F	161	1000	23,000	1.25	27.50	,,	,, (Fig. 2)
61N	F	162	500	11,500	2.5	55	Measurable, 0.5 mm lig.	,, (Fig. 6)
55N	F	163	500	11,500	1.25	27.50	Firm	Normal
$6C_3H$	M	142	500	10,000	—	—	2 mm lig.	Fig. 5
$7C_3H$	F	142	500	10,000	—	—	4 mm lig.	Fig. 1
56A	M	153	500	10,500	—	—	3 mm lig. (Fig. 3)	—

The mice were 24-75 days of age when the injections were started.

The pubes of all mice receiving testosterone and estrogen, except 61N, were firmly united at the symphysis (Fig. 4). The pelves of the mice receiving estrogens alone in similar amounts had interpubic ligaments of 3 to 5 mm (Fig. 3). The pubes of 61N were movable but were separated by a ligament of less than 0.5 mm.

Conclusion. Testosterone propionate inhibits the skeletal changes (pubic resorption and hypercalcification of the skeleton) which occur when estrogens are injected over extended periods.